Semester 1 (2023)

Membranes & Receptors Module

Membrane Structure & Dynamics

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Intended Learning Outcomes

- Understand the structure and functions of biological membranes (LO1.1)
- List the main kinds of membrane lipids, their functions and mobility (LO1.2)
- Understand the influence of unsaturated fatty acids and cholesterol on membrane fluidity (LO1.3)
- Distinguish the three major classes of membrane proteins (LO1.4)
- Know the various functions of membrane proteins (LO1.5)
- Describe the restrictions on protein movement in the membrane (LO1.6)
- Understand the specificity of erythrocyte membrane and cytoskeleton, as a model (LO1.7)
- Explain the general process of membrane formation (LO1.8)

Cell Membranes

- All cells have a membrane to separate and protect it.
- The membrane surrounding the entire cell is the **plasma membrane**.
- In Eukaryotes, cells consist of cytoplasm and organelles, all of which are surrounded by cell membranes.
- Each organelle has membrane similar to the plasma membrane's structure and function, with some differences related to their functions.

Plasma membrane functions

- Barrier and protector: Protects cell components.
- Allows nutrients and other essential elements to enter the cell, and waste materials to leave the cell, **selectively**.
- Responsible for cell's communication with its environment:
  - Recognition of signalling molecules, adhesion proteins, immune molecules.
  - Signal generation in response to electrical or chemical stimuli.

Regulation of transport across membranes

- Water moves in and out through the membrane both directly through the lipid bilayer, and through ion channels and special proteins called **aquaporins**.
- Small and non-polar molecules, such as $\mathbf{O_2}$, $\mathbf{CO_2}$, and ***some* hormones**, are able to pass freely across the lipid bilayer.
- The passage of large or polar molecules, like ions, amino acids and sugars, is regulated through membrane proteins.
- Larger materials use **endocytosis** and **exocytosis** by the formation of **vesicles**, to cross membranes.

The Plasma Membrane Components

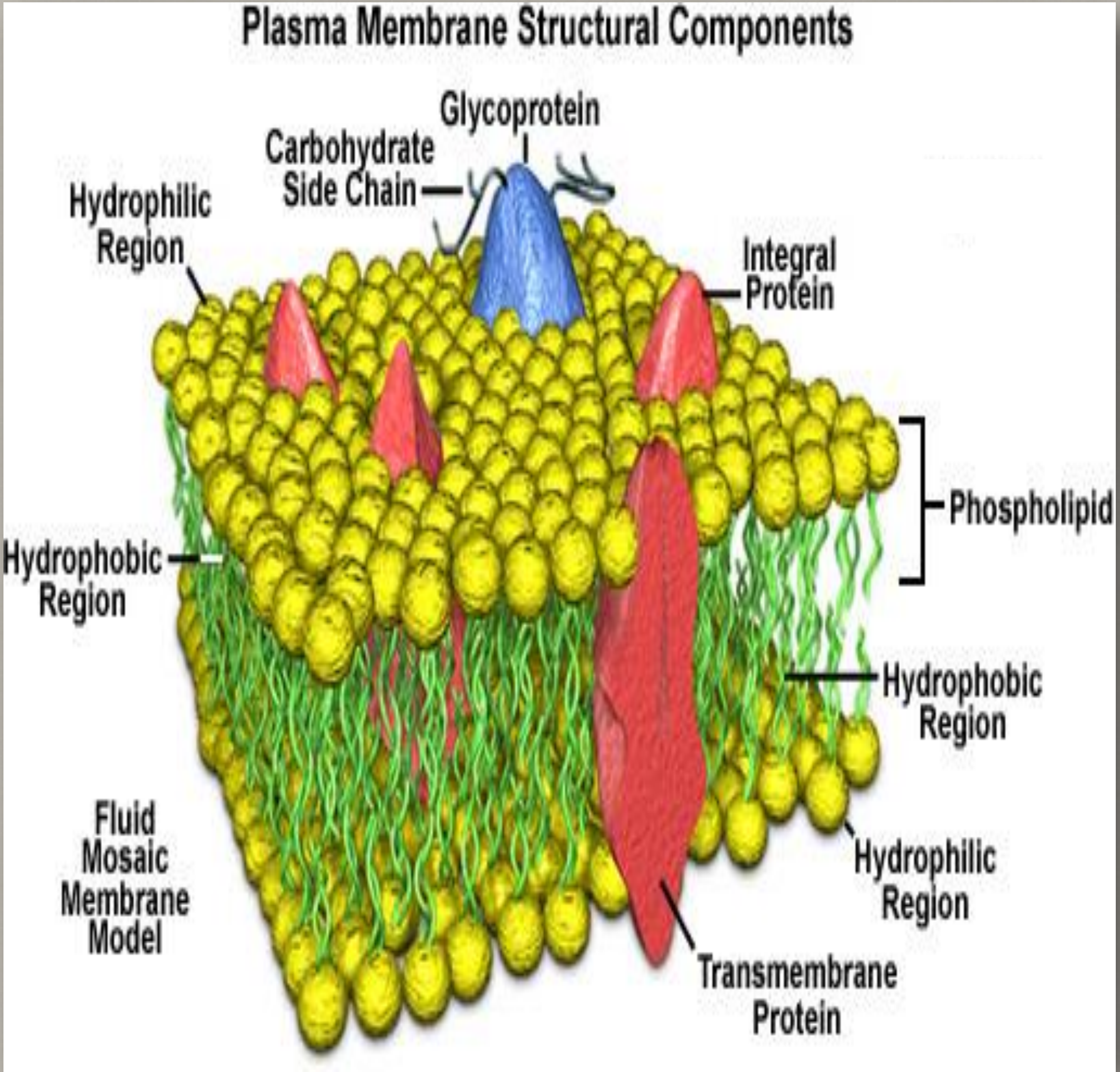
- About 5 nm thick.
- Lipid: 40%
- Protein: 60% average (18% (myelin) - 75%(mitochondria).
- Carbohydrate: 1-10%
- Water: 20%

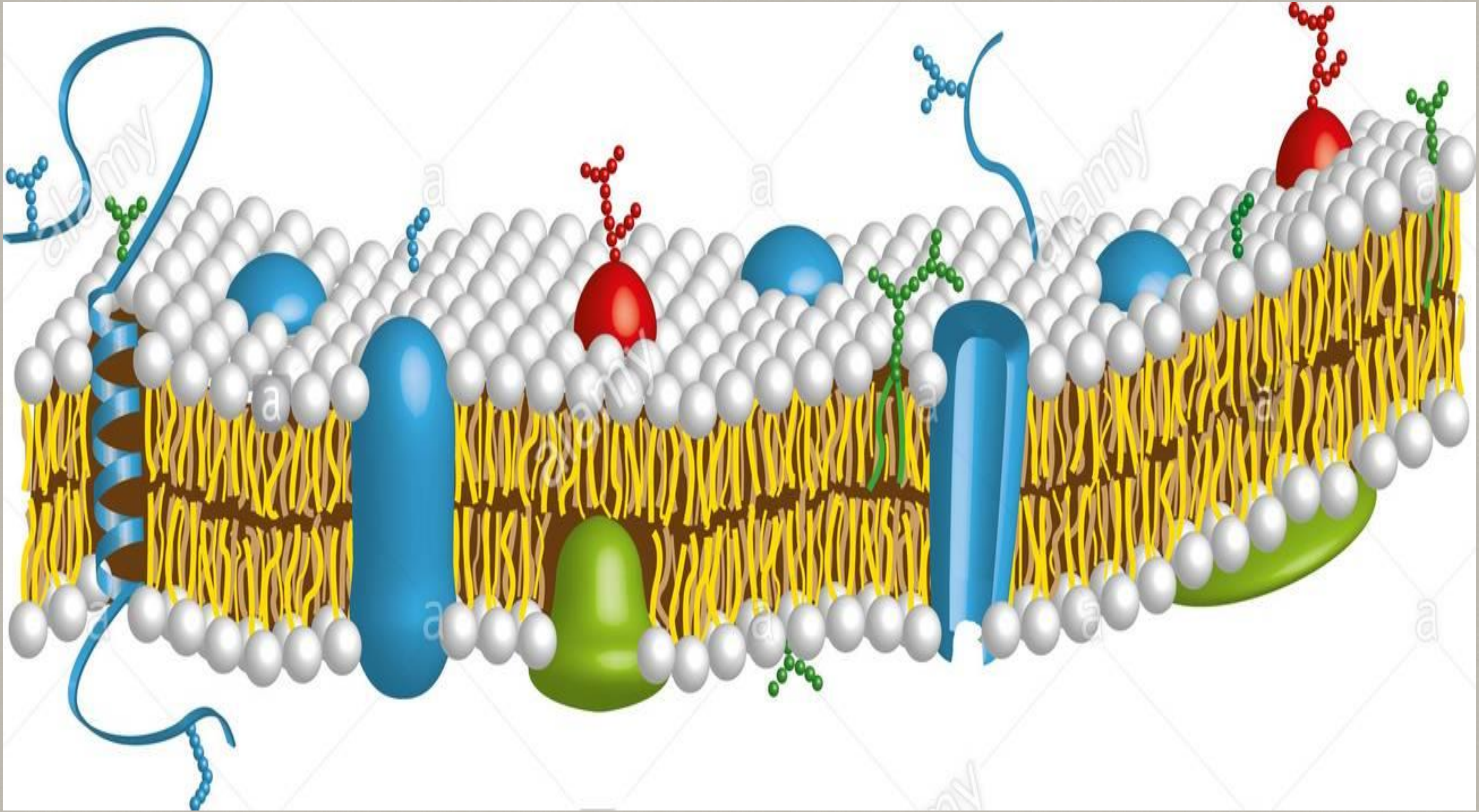
These percentages vary a little between different cells.

They are also the components of organelle membranes, with some differences.

Membrane’s Structure

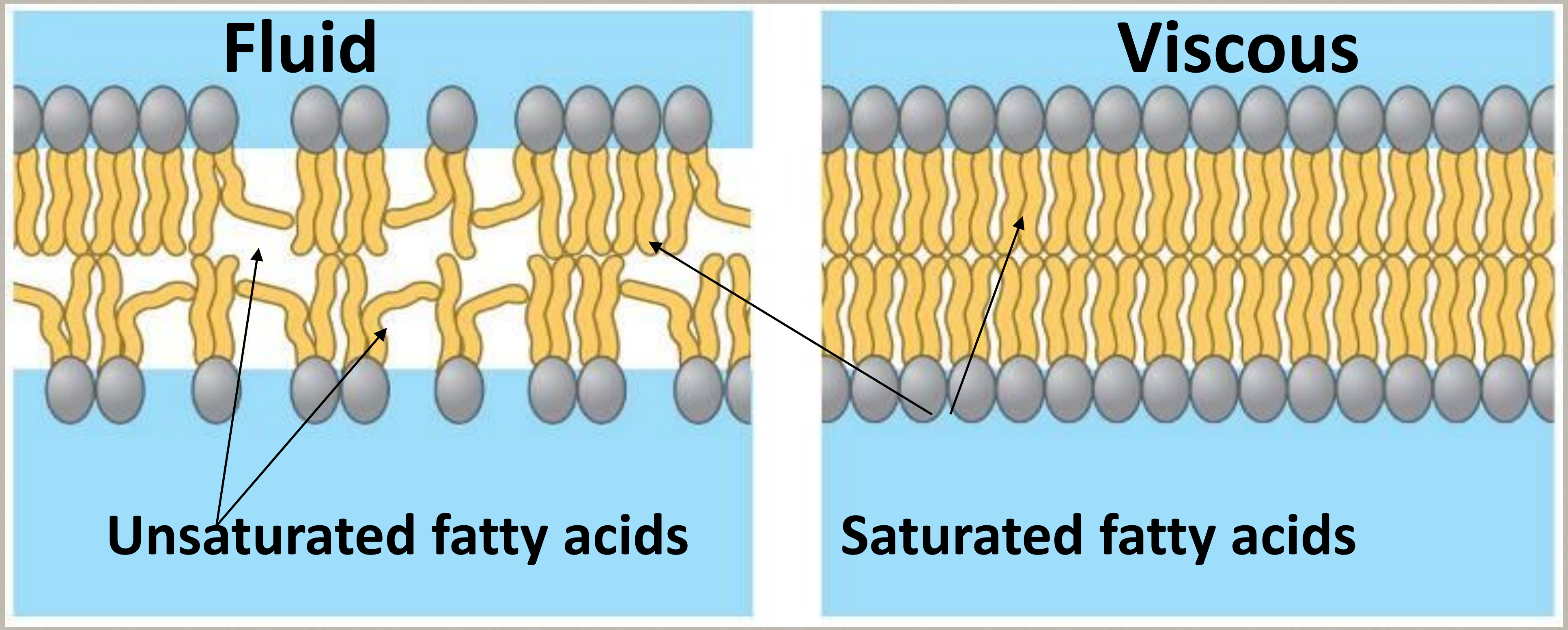
- A bilayer of **phospholipids** (**Fatty acids** with charged **phosphate head groups**), and **proteins**.
- Has a ***fluid*** structure with a “mosaic” of various proteins embedded in it (**fluid-mosaic model**).





Membrane Fluidity

- Membranes must be fluid to work properly. It involves the structure and dynamics of the lipid and protein components.
- Determining and regulating the fluidity is mainly through unsaturated fatty acids, and cholesterol.



Membrane lipids

1. **Phosphopholipids:**

A. Phosphoglycerides: Derived from glycerol-three-phosphate (G-3-P) fatty acids (saturated and unsaturated). Types:

1: Phosphatidylcholine (PC); major type.

2: Phosphatidylserine (PS).

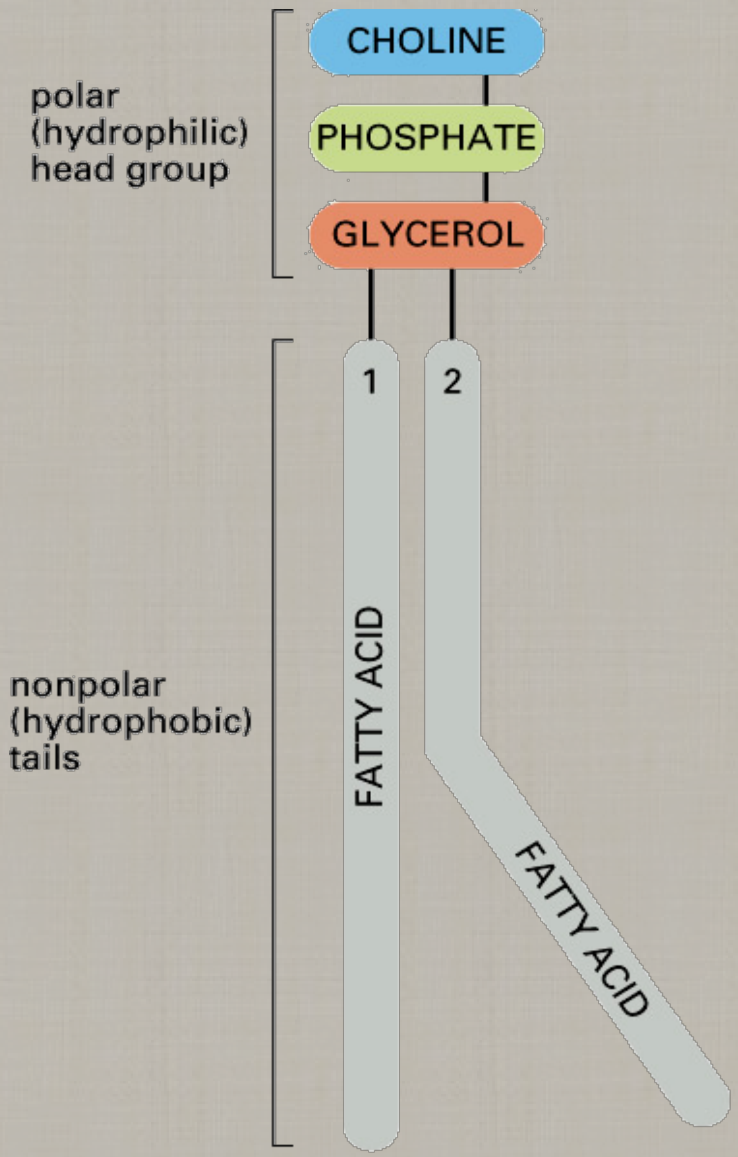
3: Phosphatidylethanolamine (PE)

4: Phosphotidyleinositol (PI)

B. Sphengomyelin (SM): No glycerol in the structure.

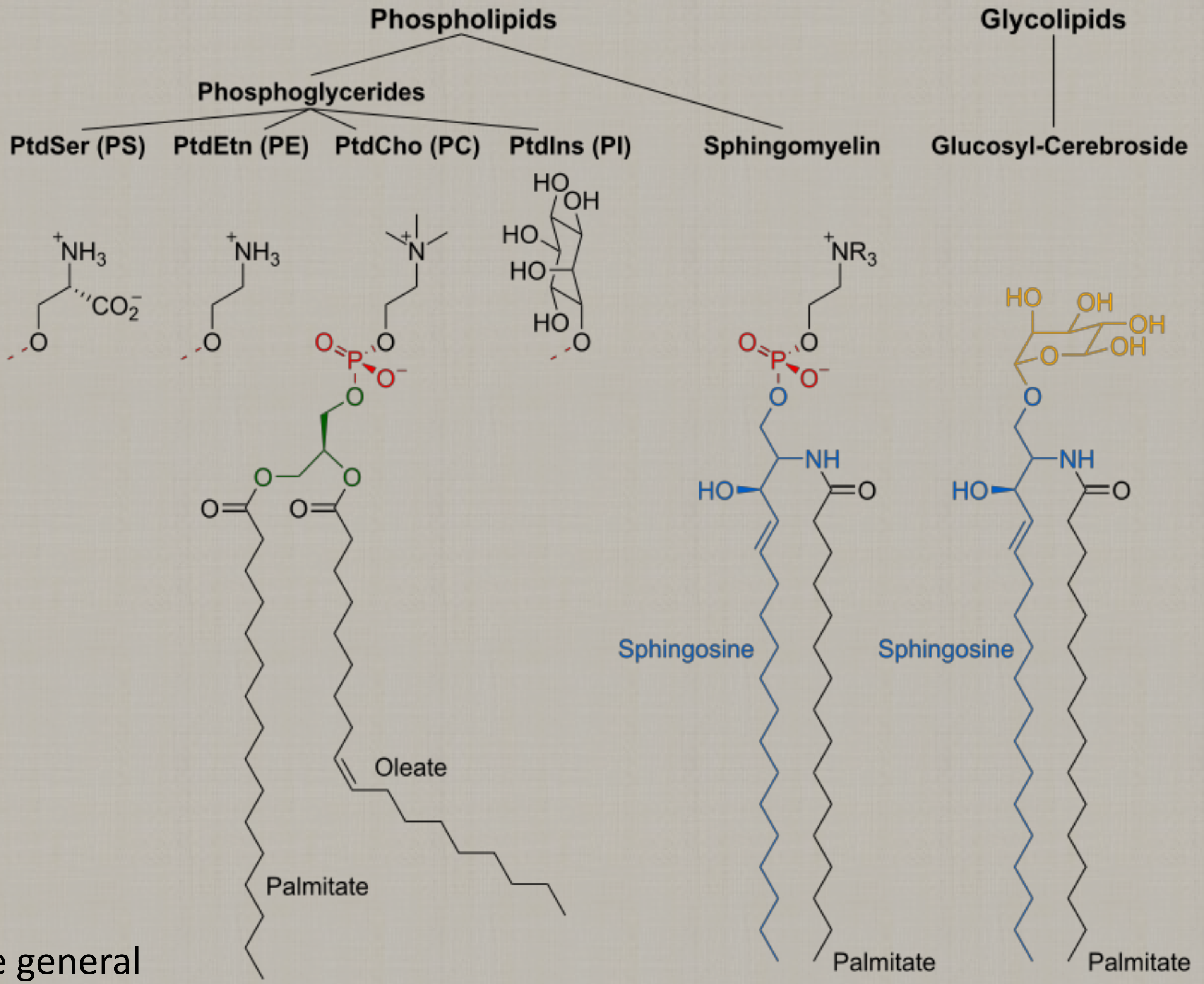
2. Glycolipids

3. Cholesterol (animal cells. In plants, Stigmasterol replaces it).



Phosphatidylcholine , as an example of the general structure of a phosphoglyceride

Students are required to learn this structure.

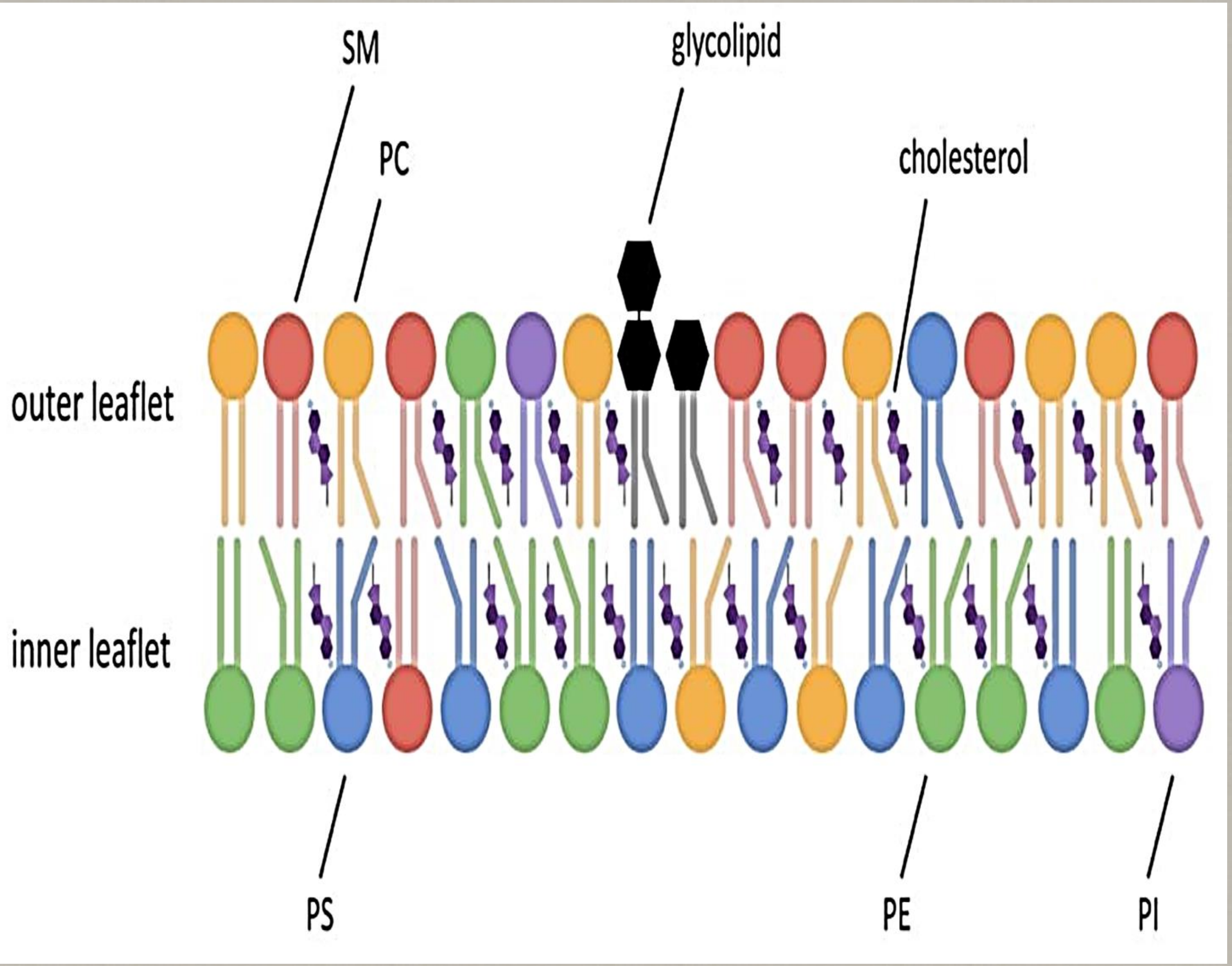


Different types of phospholipids

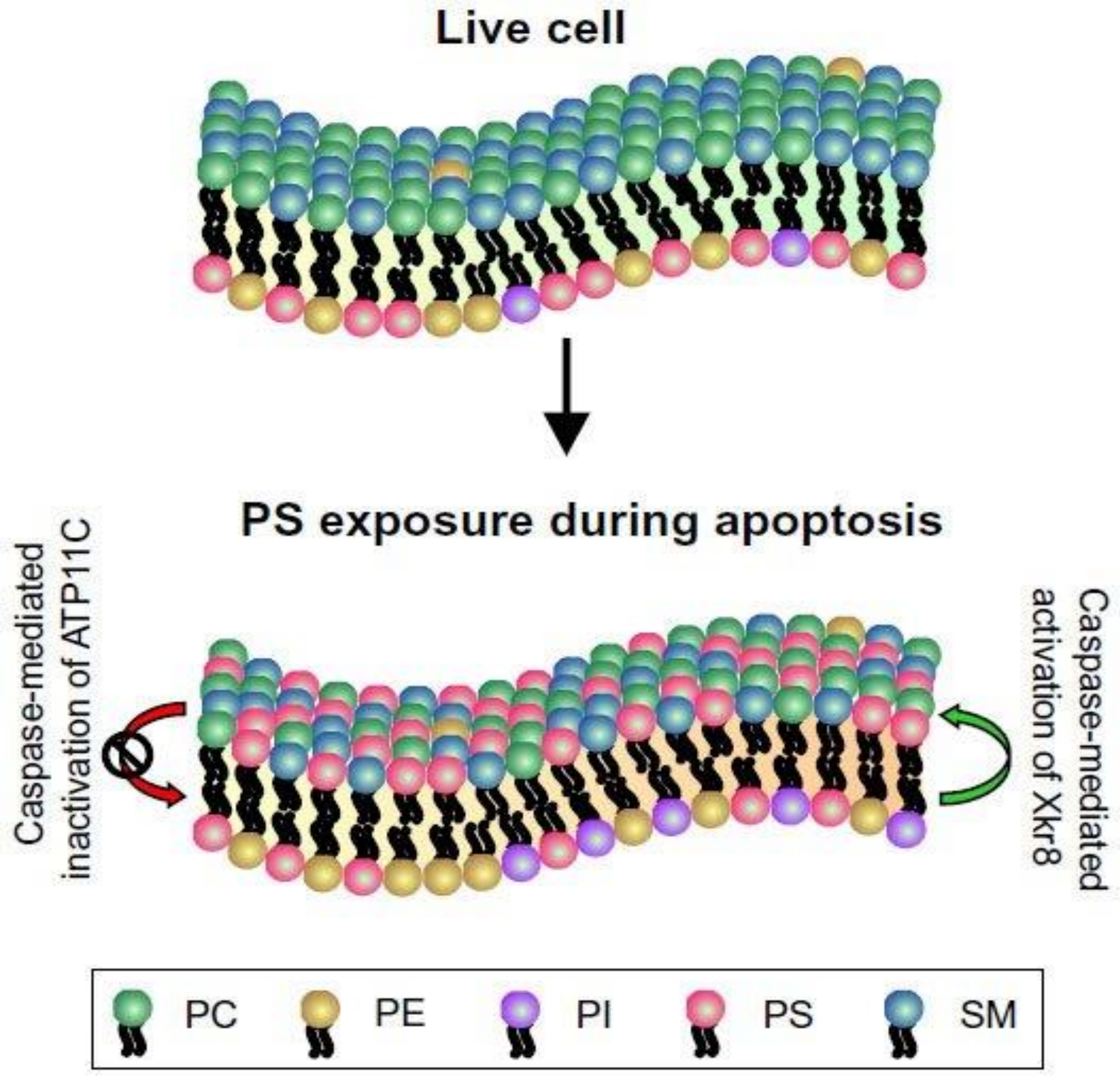
Students are NOT required to learn this structure.

Membrane lipid asymmetry

- Phosphatidylcholine (PC) and sphingomyelin are mainly localized in the extracellular leaflet.
- Phosphatidylethanolamine (PE) and phosphatidylserine (PS) are present almost entirely in the inner leaflet.

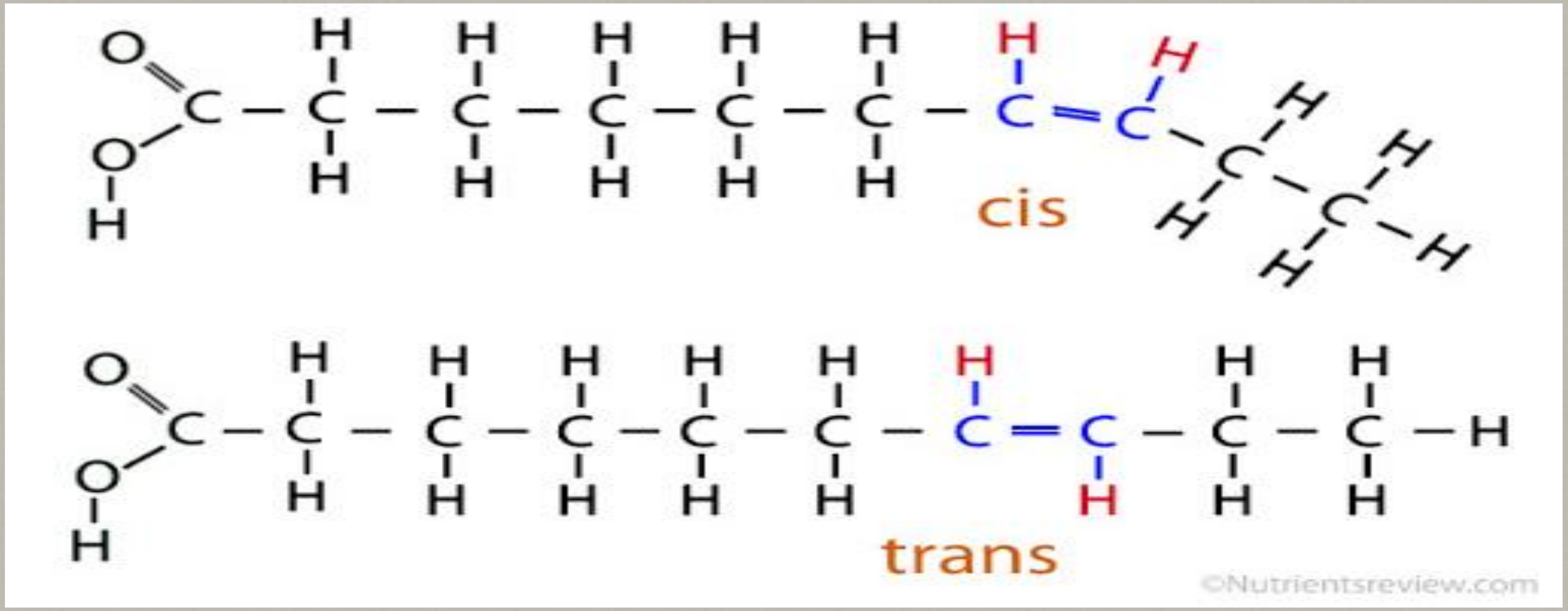


- Maintenance of the lipid asymmetry requires carrying **flip-flop** *actively* by enzymes.
- This is important for a variety of cell processes. Example: The movement of PS to the outer leaflet is a step of **apoptosis**. Exposed PS are recognized by macrophages to engulf the apoptotic cells.

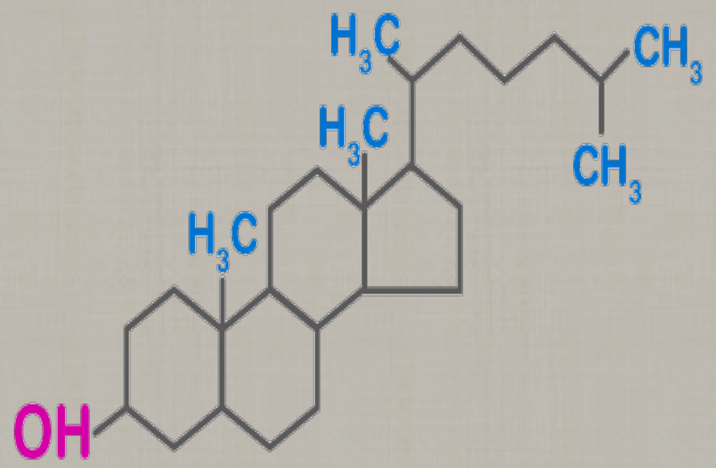


Saturated and unsaturated fatty acids

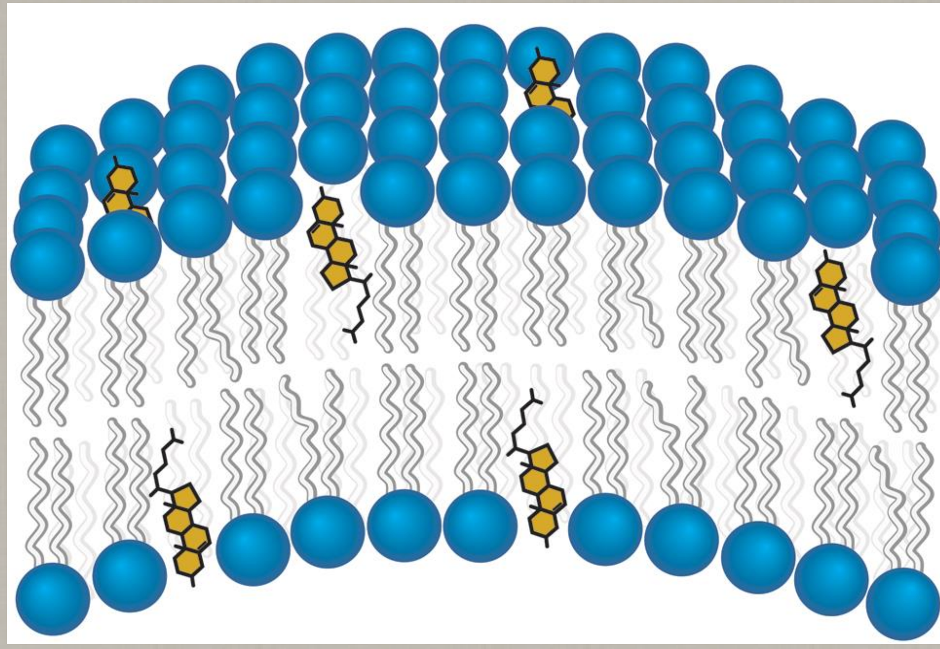
- Membrane fatty acids are a mixture of saturated and unsaturated.
- The membrane unsaturated fatty acid chains are in the ***cis*** conformation (hydrogen atoms adjacent to double bond are on the same side). This makes a **kink** in the chain which reduces phospholipid packing and **increase** fluidity.



Membrane's Cholesterol

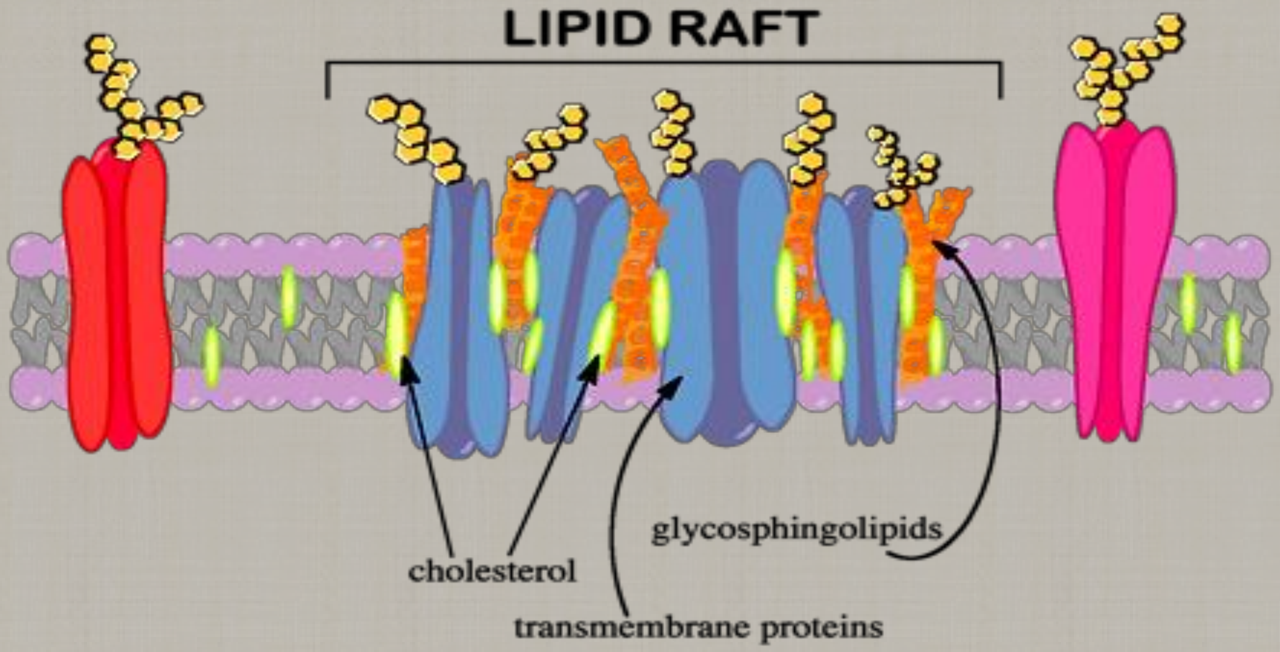


- Provides rigidity and mechanical stability to the plasma membrane.
- Regulates fluidity.
- Lowers permeability, and affects water penetration.
- Generates natural negative curvature in lipid bilayers.
- Involved in exocytosis (mostly neuronal and endocrine cells).
- An antioxidant that protects the membrane against radiation-initiated oxidation.



- Involved in **raft formation**: Organised structures of protein receptors, cholesterol, and glycosphingolipids. Cholesterol seem to be the glue that holds the raft together.

- The rafts float freely within the membrane bilayer, and seem to serve as organising centers for signaling molecules, allowing a closer interaction of receptor proteins and their effectors.

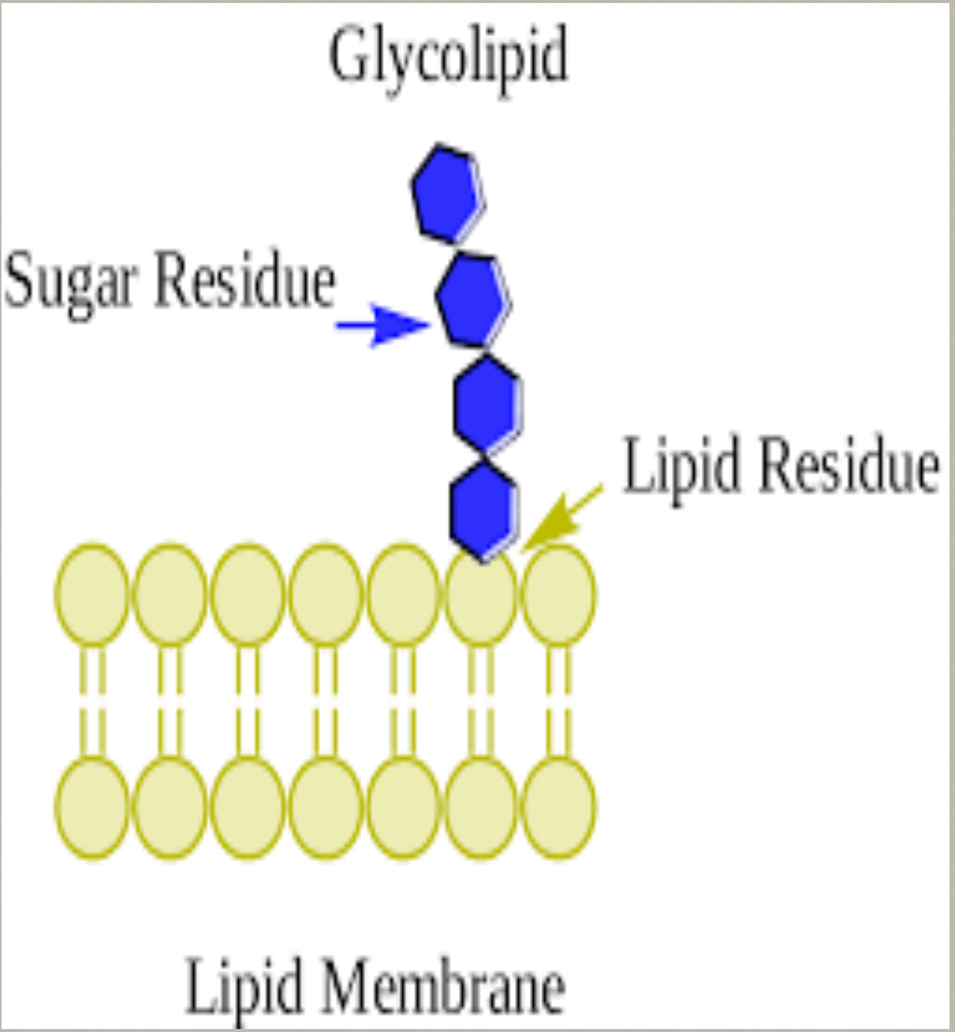


Membrane Fluidity regulation by cholesterol

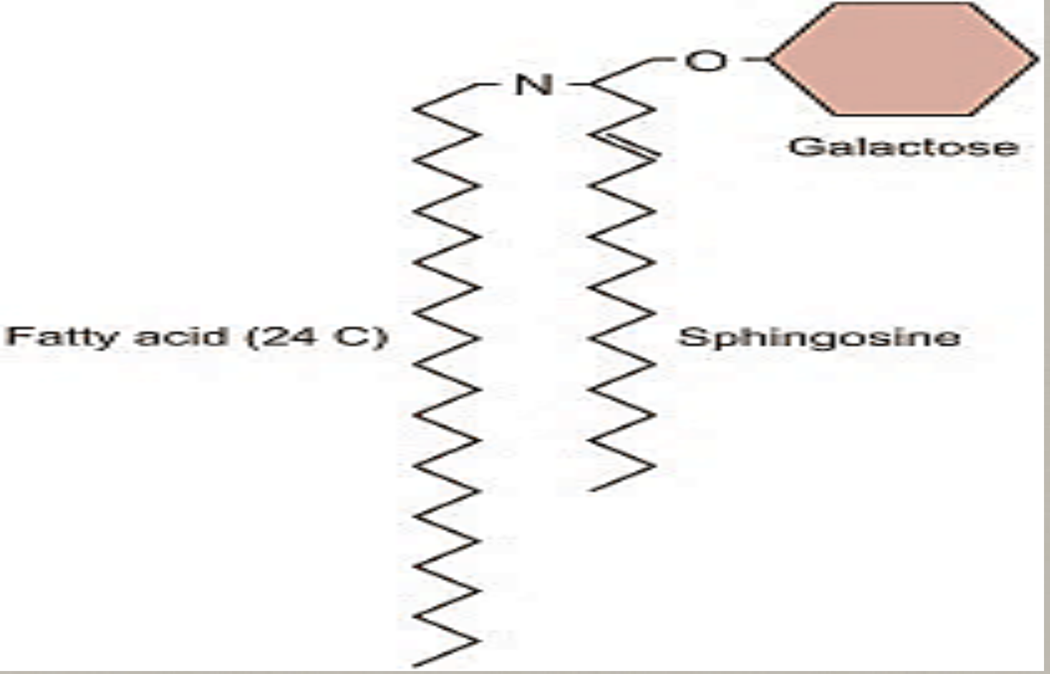
- ◆At high temperatures, it stabilizes the membrane and lowers fluidity.
- ◆At low temperatures it intercalates between the phospholipids and prevents them from clustering together and stiffening, therefore increasing fluidity.
- ◆Ability to change the degree of the fluidity in response to temperature changes, has evolved in organisms that live where temperatures vary.

Membrane's Glycolipids

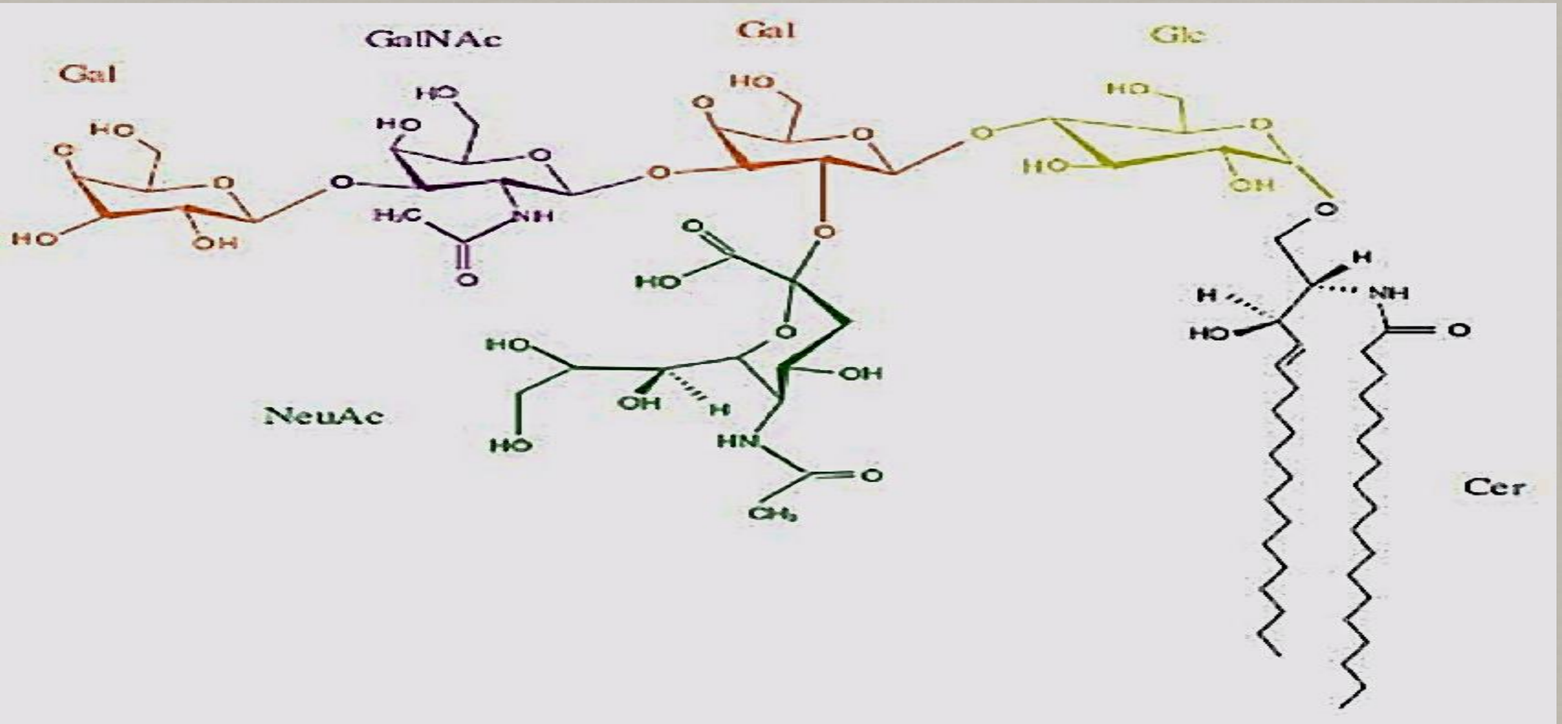
- Composed of carbohydrates attached to lipids.
- Important in membrane stability, cell-cell recognition, crucial to the immune response, in tissue formation, and are important in **signal transduction**.



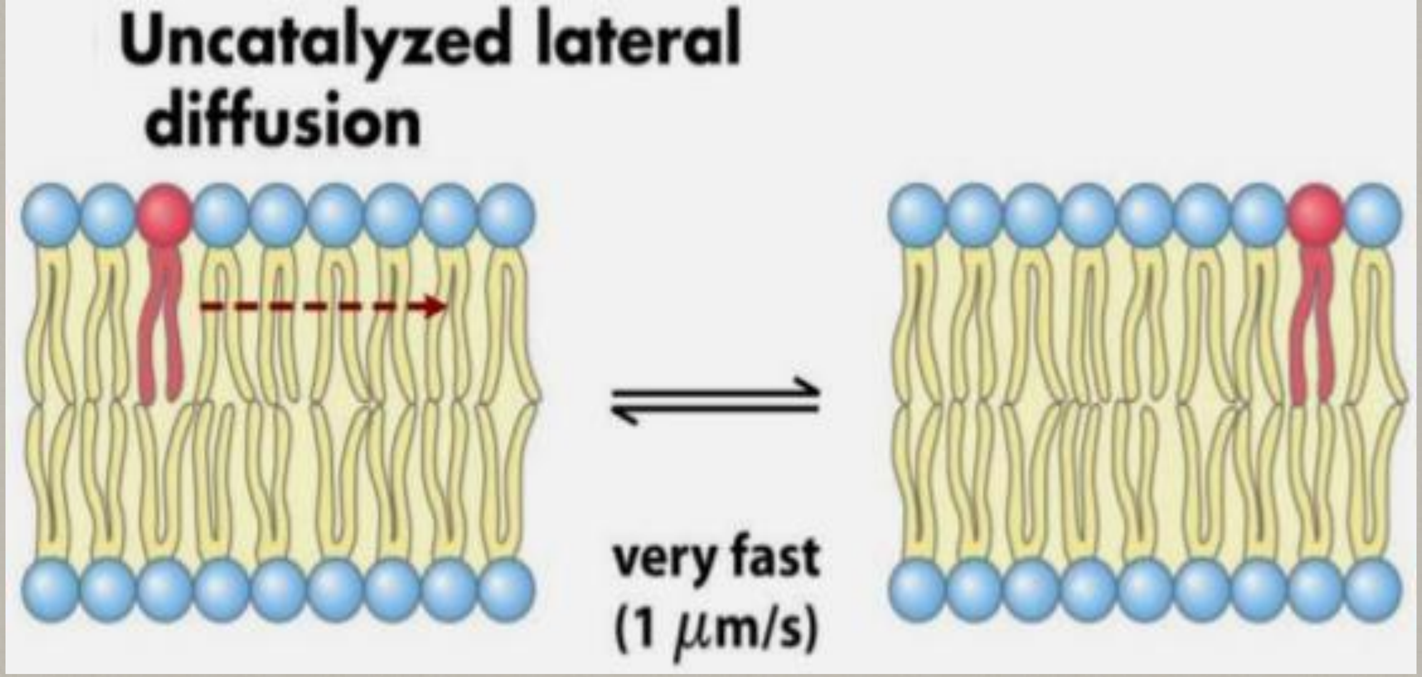
1.Cerebrosides - head group sugar monomers



2. Gangliosides - head group oligosaccharides

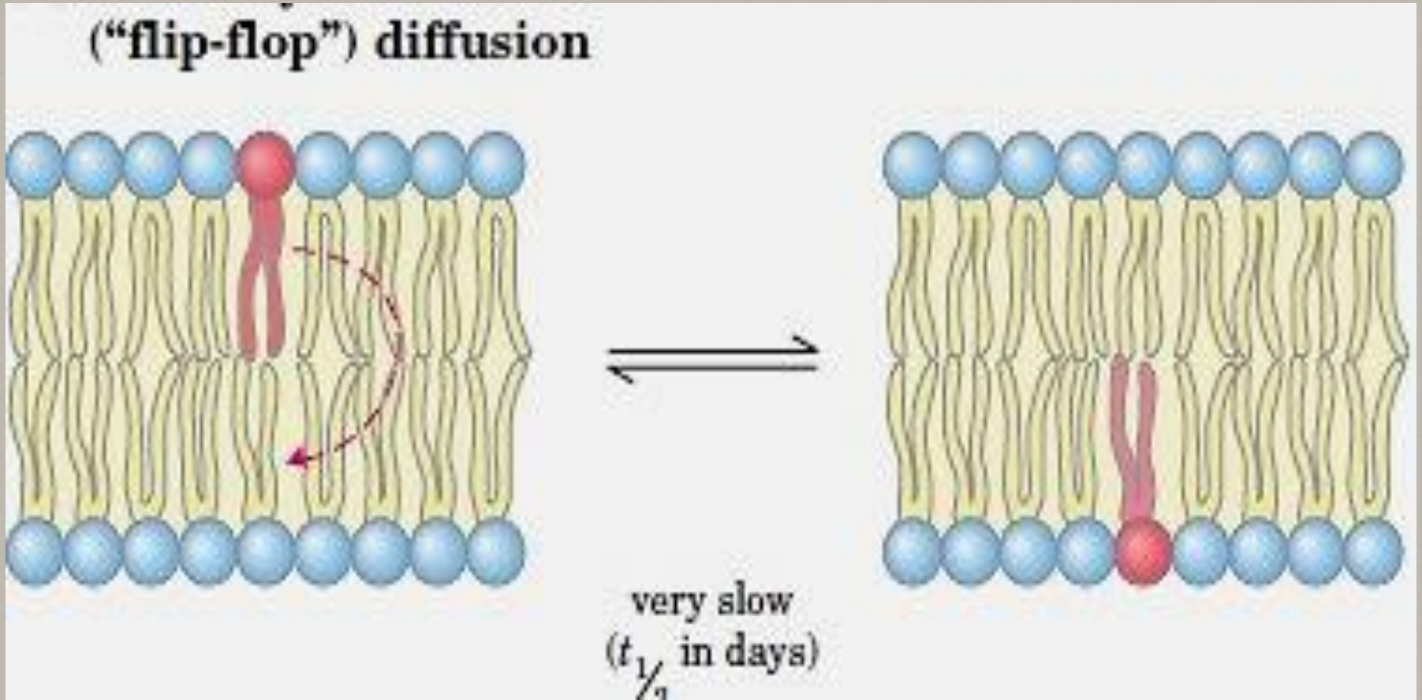


Dynamics in lipid bilayers: 1- Fast lateral diffusion within the plane of the bilayer.



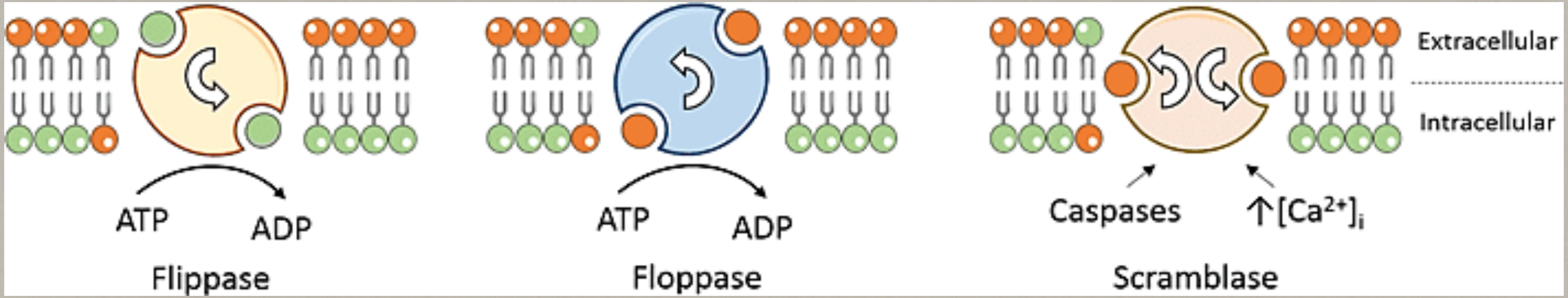
Dynamics in lipid bilayers: 2- Flip-Flop movement.

Exchange of lipid molecules from one half of the bilayer to the other.

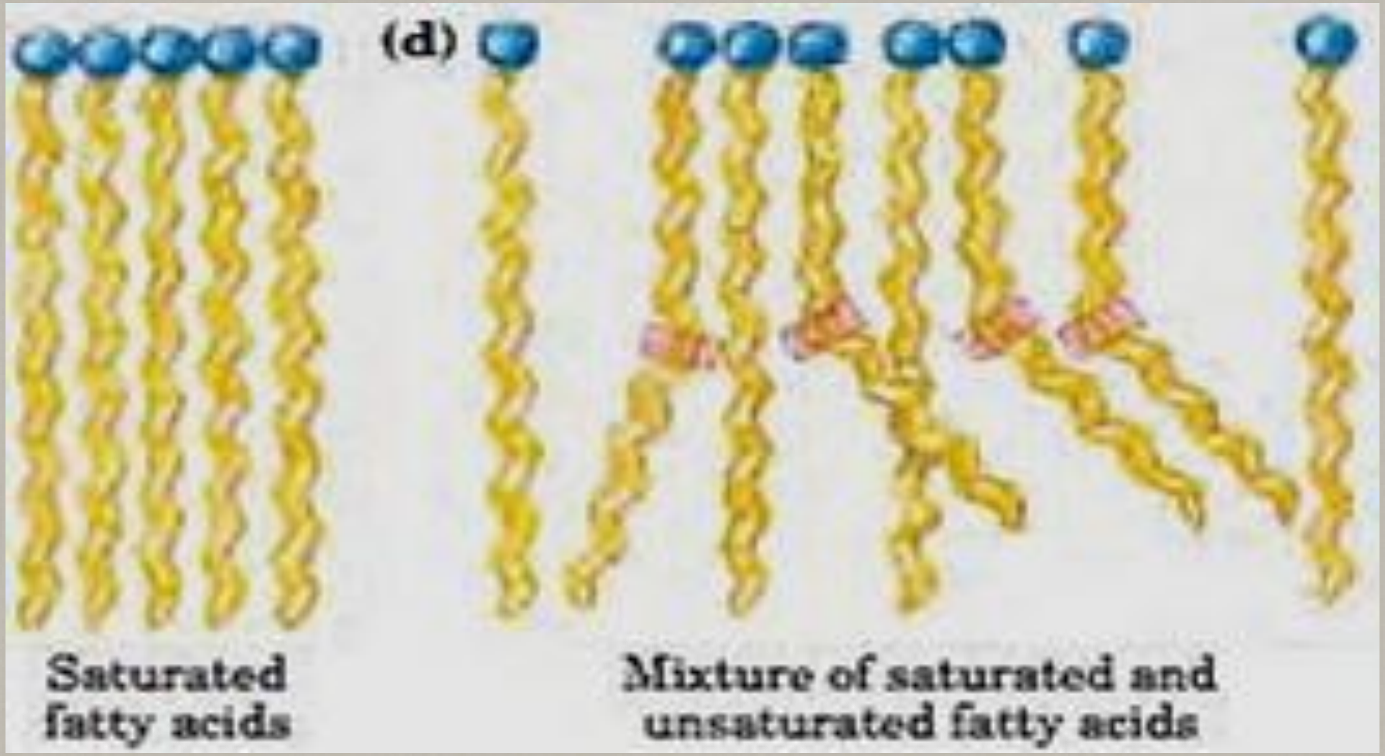


Flip-flopping through enzymes

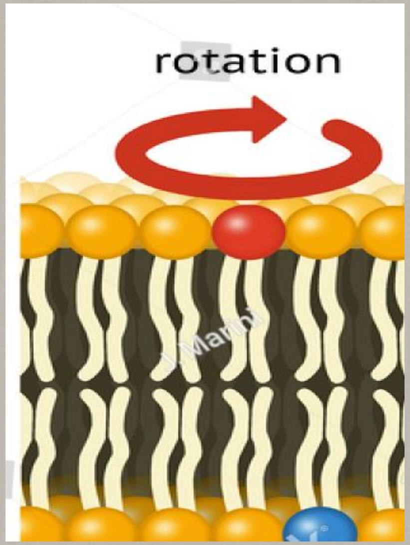
- **Scramblase:** Translocates phospholipids between the two layers. Does not need ATP and is calcium dependent.
- **Flippase:** Moves an outer phospholipid leaflet to the inner leaflet. Needs ATP.
- **Floppase:** Functions in the opposite way. Needs ATP.
- Flippases and floppases equalize the number of lipids at both sides of the membrane when a new membrane is generated.



Dynamics in lipid bilayers: 3- Intra-chain motion - kink formation in the fatty acyl chains



Dynamics in lipid bilayers: 4- Fast axial rotation



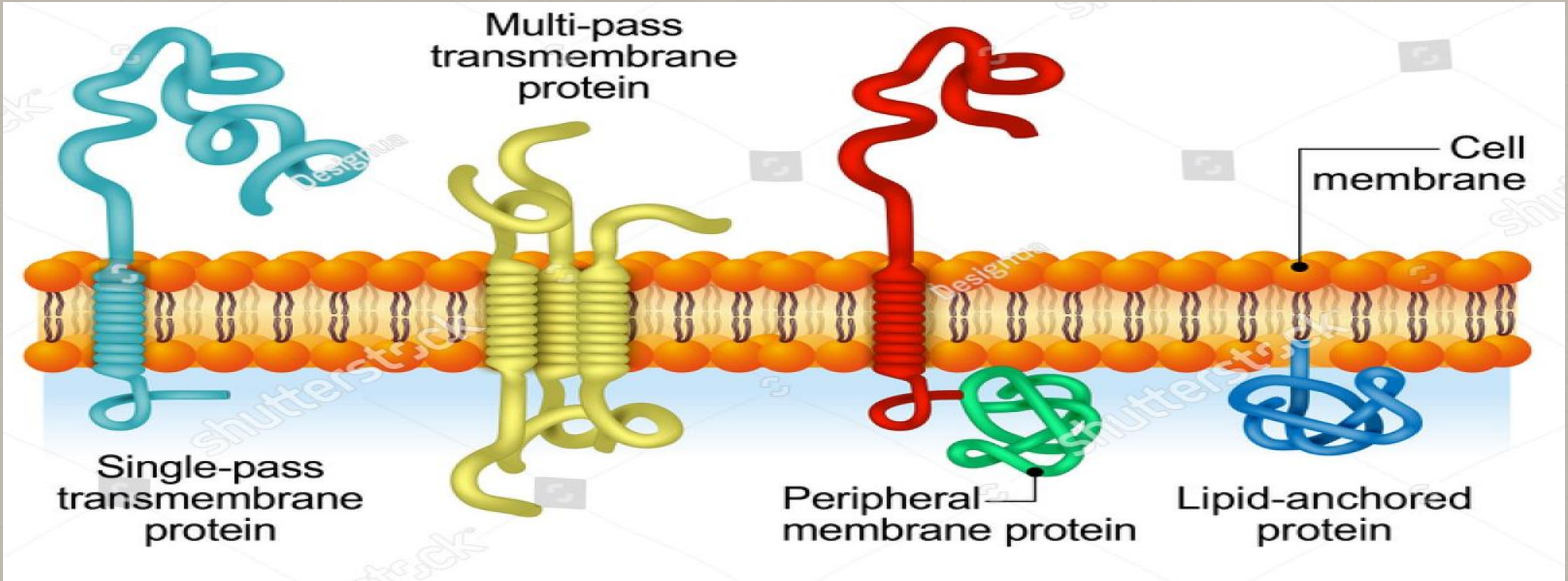
Membrane Proteins

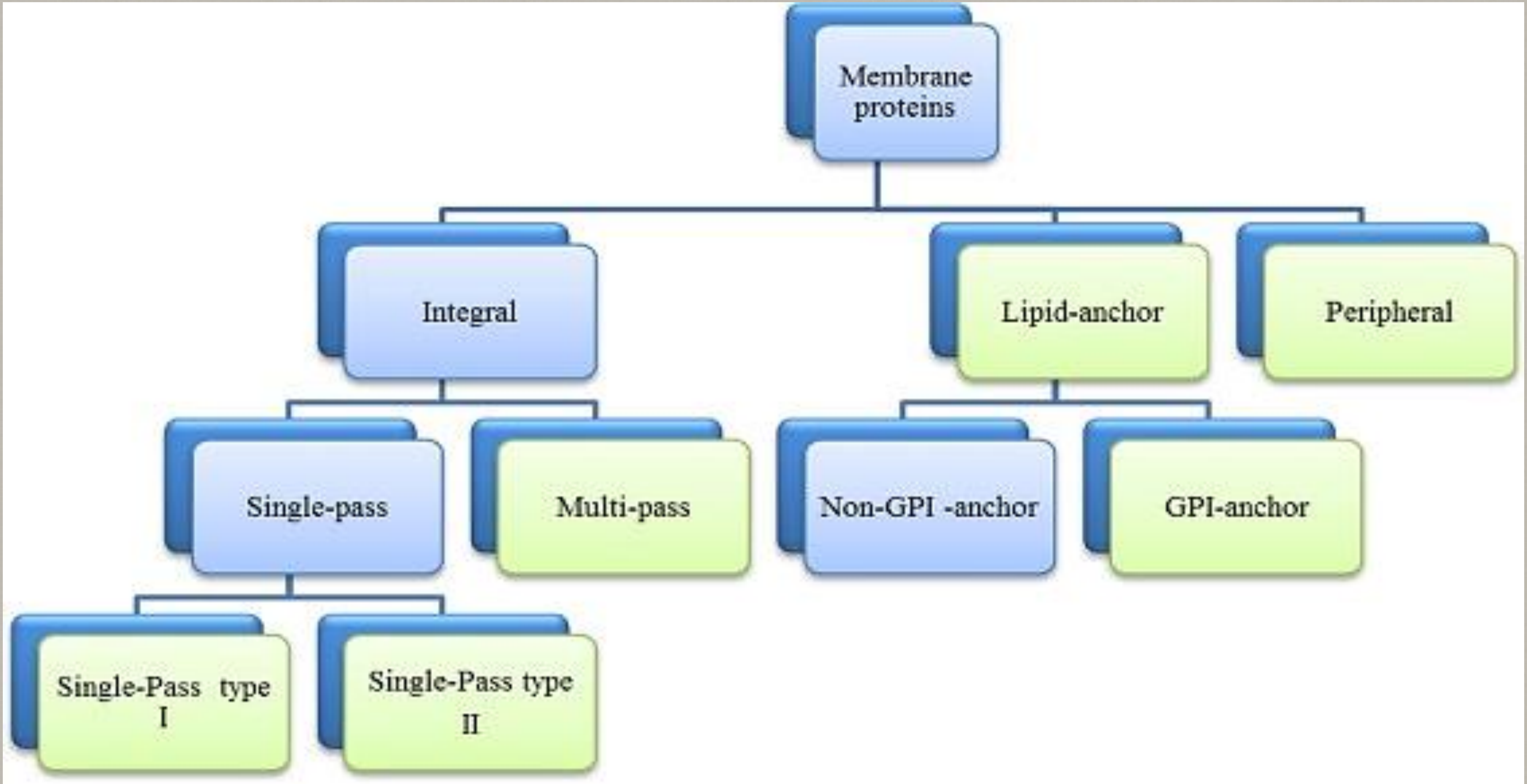
- There are many types of membrane proteins and they determine most of the membranes’ functions.
- Based on their position, they are classified into: **Integral**, **Peripheral**, and **Lipid-anchored**

Integral: Embedded in the lipid bilayer. Some of them span the entire membrane and are called **transmembrane** proteins.

Peripheral: Associated with integral membrane proteins, or membrane's lipids, temporarily.

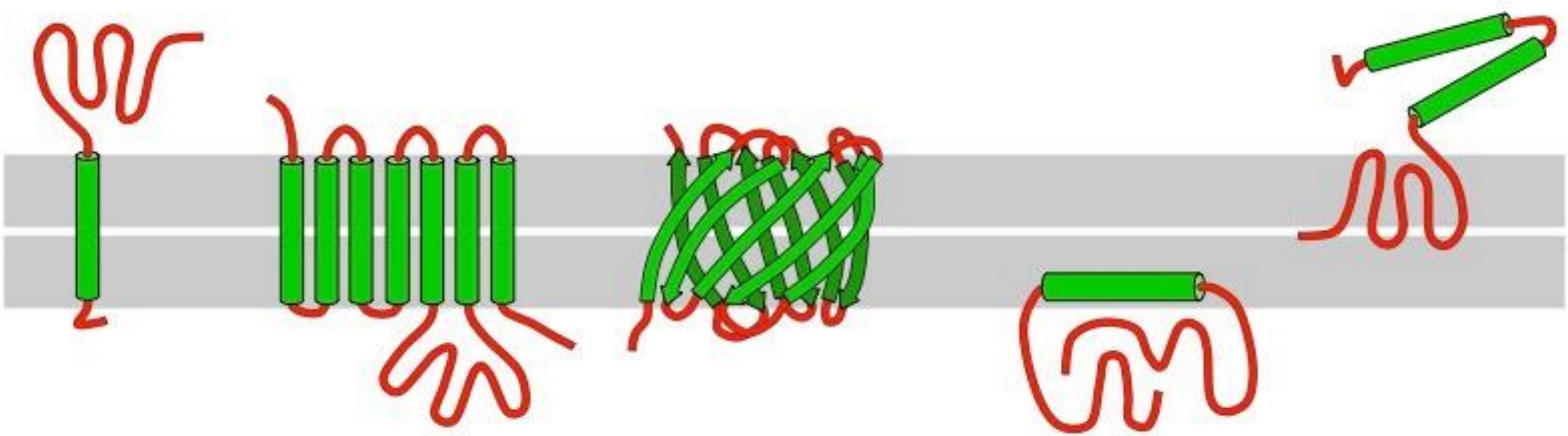
Lipid-anchored: Covalently attached to lipids of the cell membrane.





GPI: Glycosylphosphatidylinositol (GPI) is a lipid anchor for many cell-surface proteins.

Integral Proteins | **Peripheral Proteins**



Integral Proteins			Peripheral Proteins
α- helix Recognition, Receptors	**Helical bundle** Enzymes, Transporters, Receptors	**β- barrel** Transporters (channel proteins)	Enzymes, Anchorage, Transporters (carriers)

Glycoproteins

- **Glycoproteins** are proteins which contain oligosaccharide chains covalently attached to amino acid side-chains.
- The attaching process is called **glycosylation**, a type of posttranslational modifications.
- The two most common glycoproteins are:

➢N-glycosylated: Sugars are attached to nitrogen, typically on the side-chain of **asparagine**.

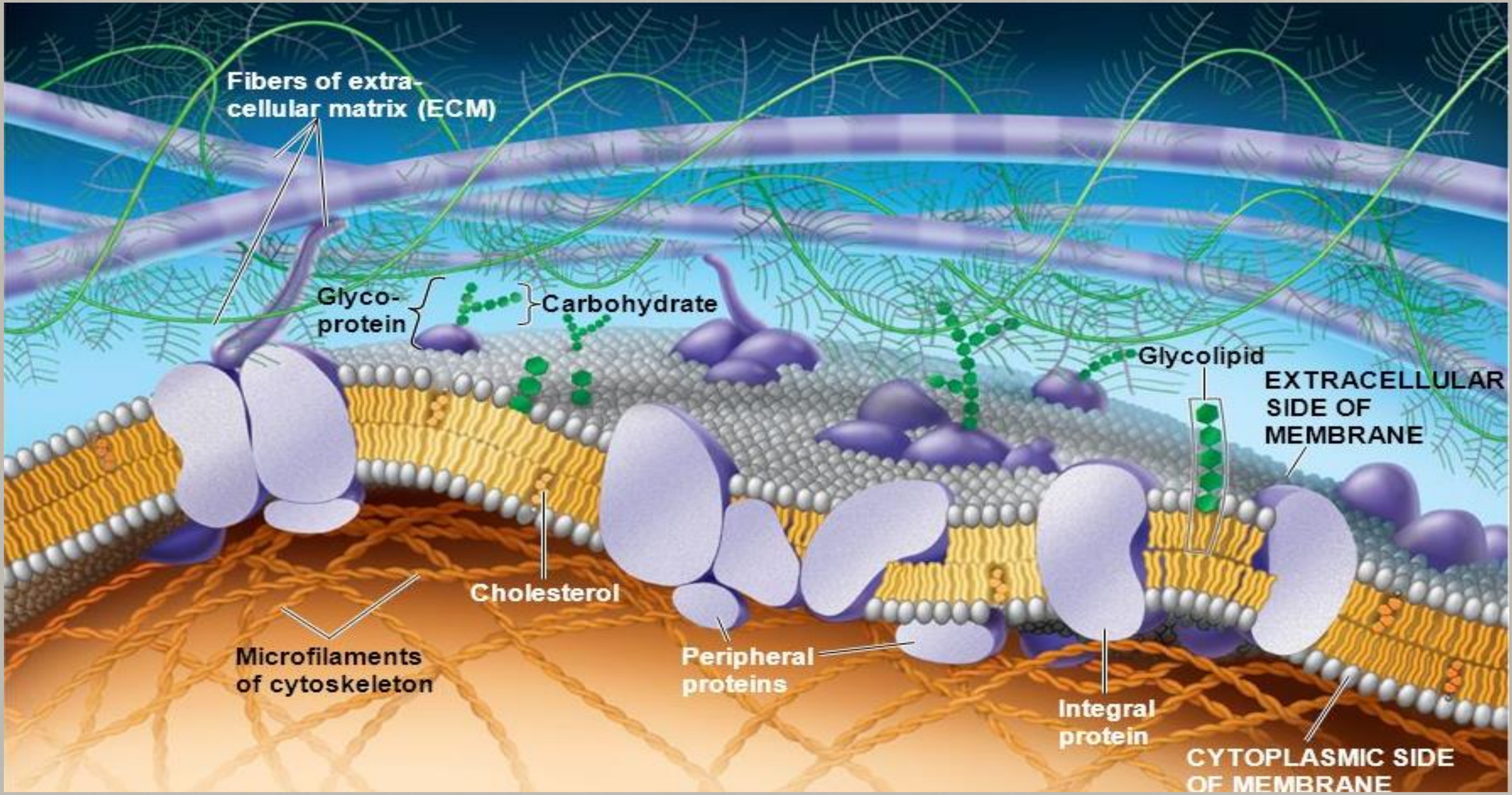
➢O-glycosylated: Sugars are attached to oxygen, typically on **serine** or **threonine**.

- Most integral proteins have α-helices structures, their hydrophobic regions consisting mostly of nonpolar amino acids.
- Membrane proteins interact with **cytosolic** and **external** protein.
- Some membrane proteins provide extra strength to the plasma membrane by attaching to proteins of the exterior surface like the **extracellular matrix (ECM)**, and on the inside part with **cytoskeletons**. In this way cells are linked together and tissue integrity is maintained.

The Cytoskeleton: A network of cytoplasm long fibrous protein skeleton structure; with other small proteins, and attached to the cell membrane.

- Three types: **Microtubules** (Important in cell division), **Intermediate filaments** (provides cell mechanical strength) and **Actin filaments** (generate contraction force for movement).

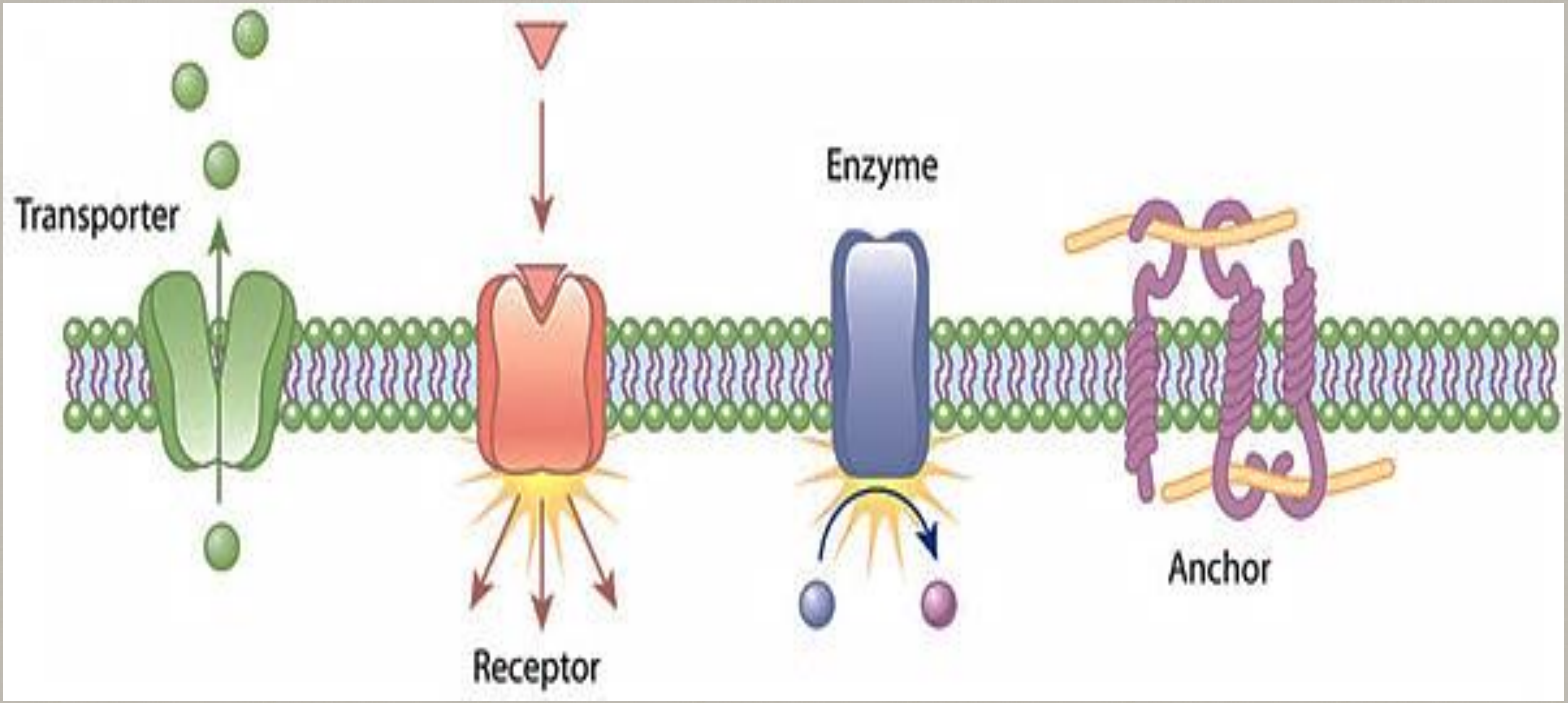
The Extracellular matrix (ECM): Is a network of proteins like collagen, enzymes and **proteoglycans** that provide structural and biochemical support to surrounding cells in connective tissues.

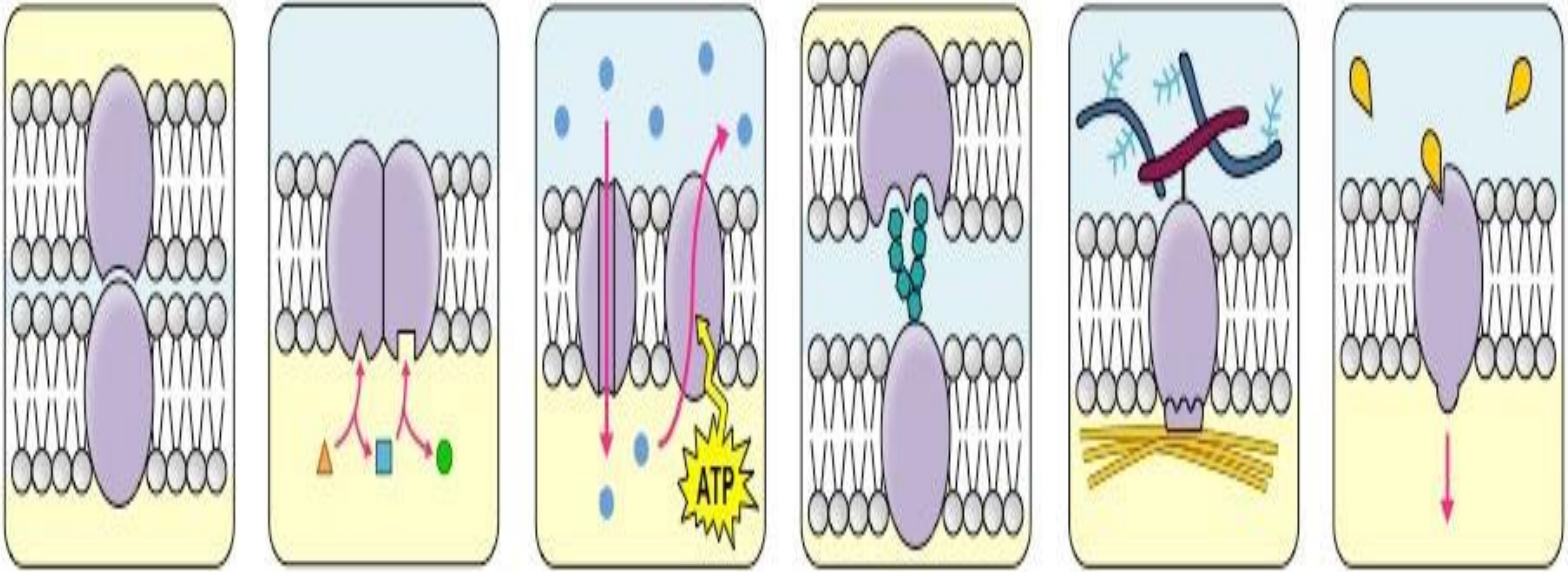


Fibers of extra-
cellular matrix (ECM)
Glyco-
protein
Carbohydrate
Glycolipid
EXTRACELLULAR
SIDE OF
MEMBRANE
Cholesterol
Microfilaments
of cytoskeleton
Peripheral
proteins
Integral
protein
CYTOPLASMIC SIDE
OF MEMBRANE

Membrane proteins functions

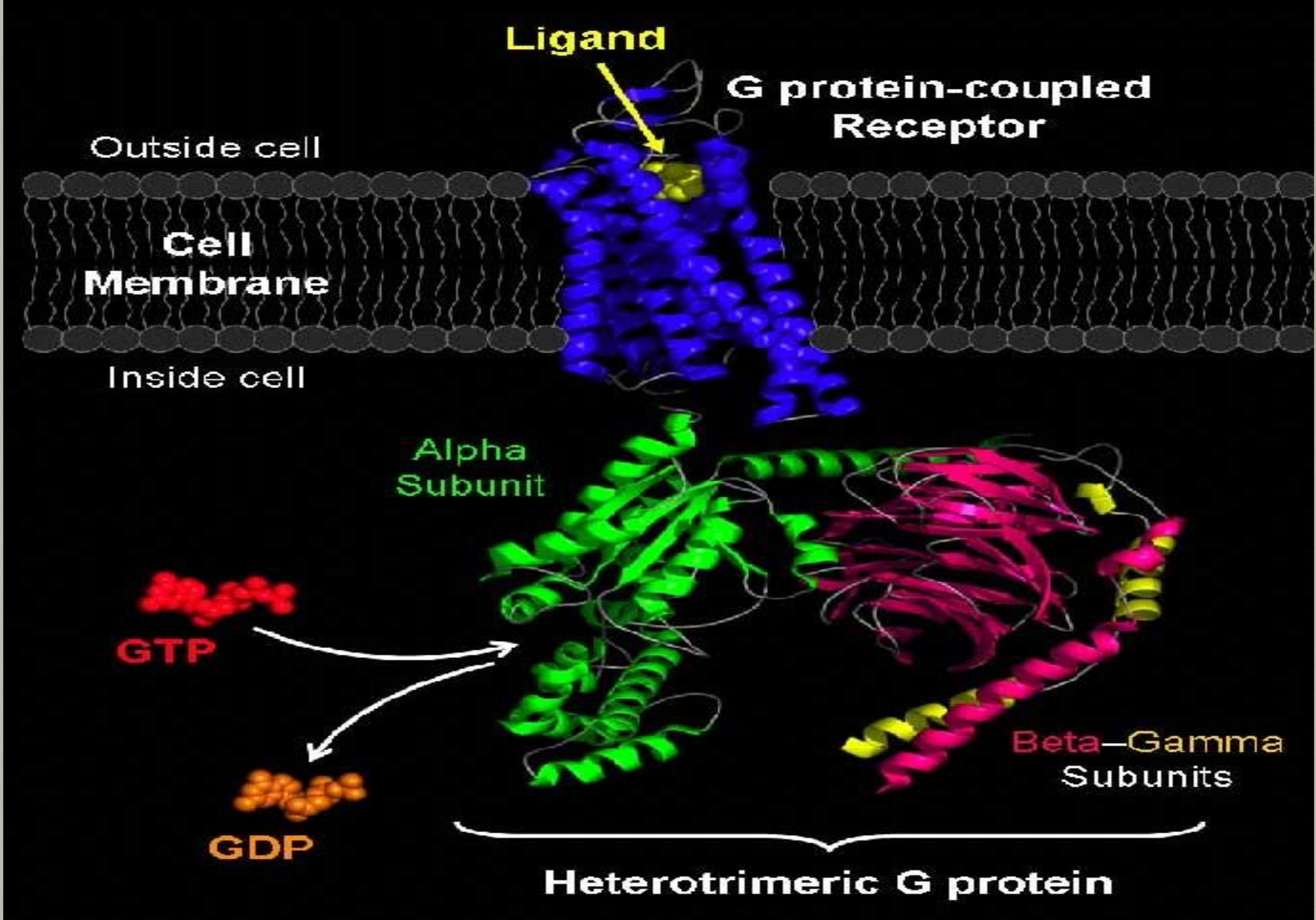
- Enzymes (example: Kinases).
- Transporters: Include **Ion channels** (Na^+, K^+, Cl^- ion channels), and **Carrier proteins** (example: The sodium-potassium pump, and Glucose transports).
- Receptors (example: Insulin receptor, growth factor receptors, Rhodopsin (light receptor protein of the retinal rod cells)).
- Energy transducers (example: Cytochromes.)
- Adhesion/Cell-cell recognition proteins (example: Integrins).


Transporter
Receptor
Enzyme
Anchor



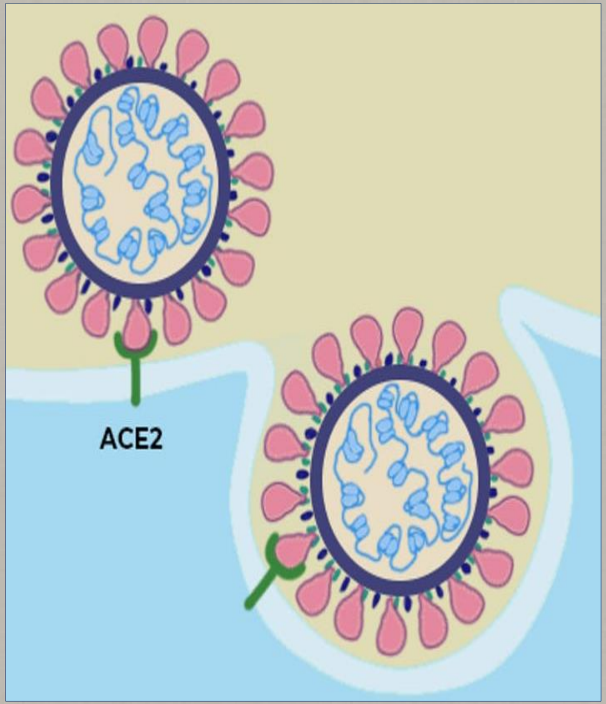
Intercellular Joinings | Enzymatic Activity | Transport (Active / Passive) | Cell-Cell Recognition | Anchorage / Attachment | Signal Transduction

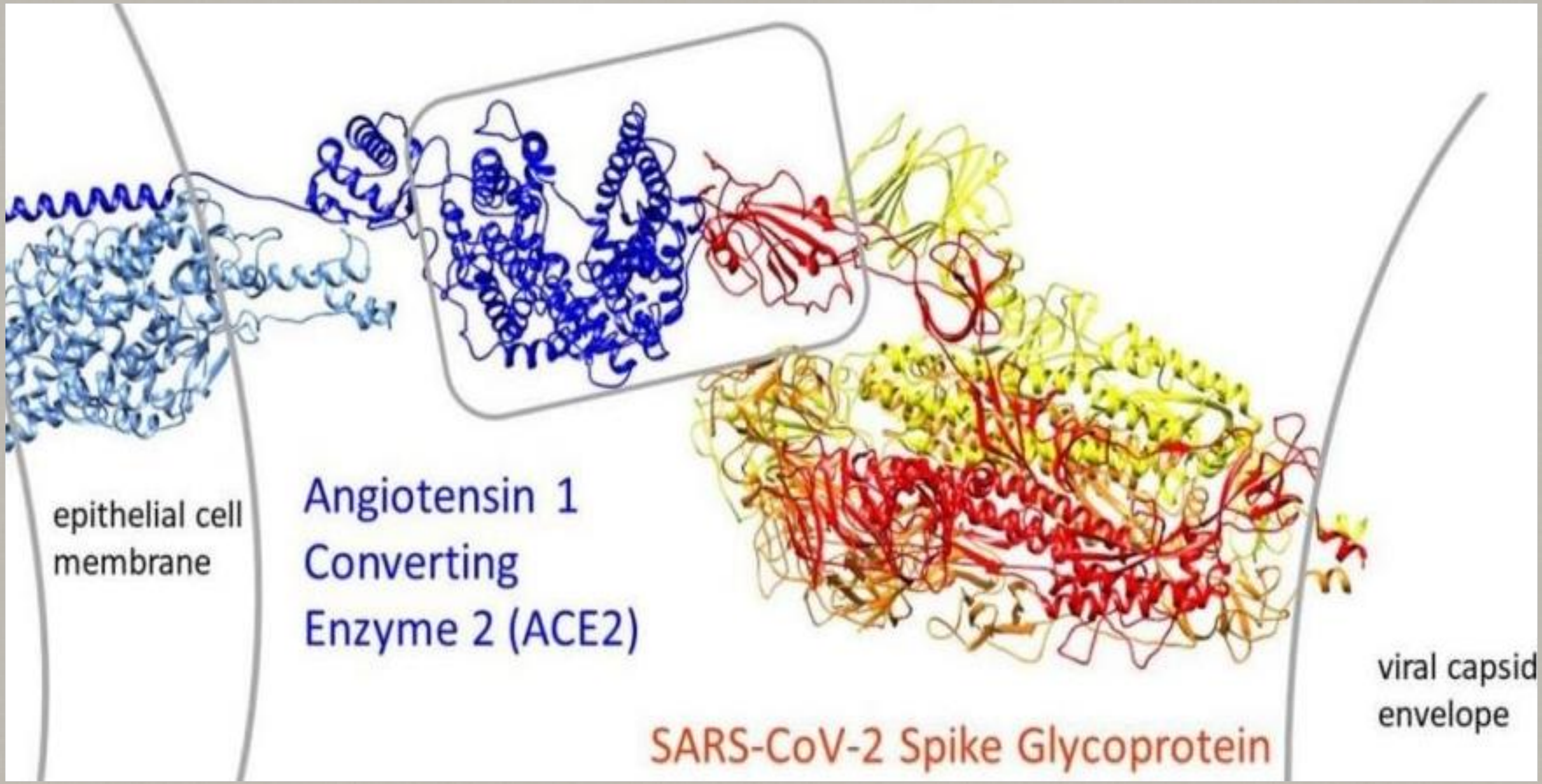
Example:
Rhodopsin (transmembrane integral protein) in retinal rod cells membranes



Example: Angiotensin-converting enzyme 2 (ACE2) is a human lung epithelial cell transmembrane protein which Covid-19 viral **Spike Glycoprotein** interacts with, to attach and enter the cells.

ACE2 normally binds **angiotensin II** peptide hormone (has a role in blood pressure regulation).



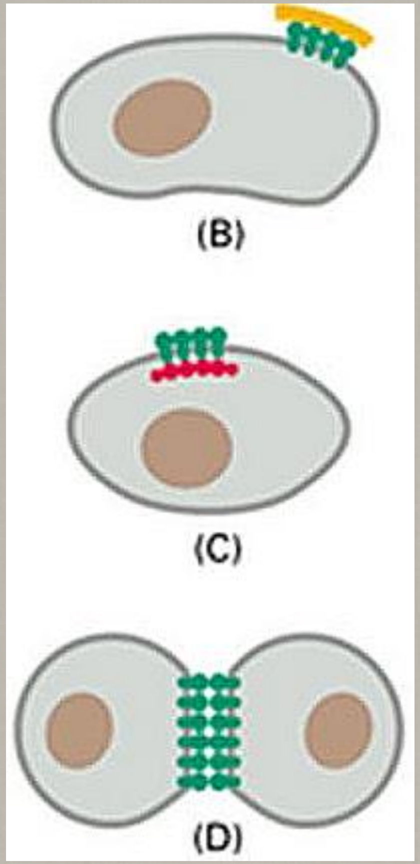


Mobility of membrane proteins

1. **Conformational change**
2. **Rotational**
3. **Lateral**

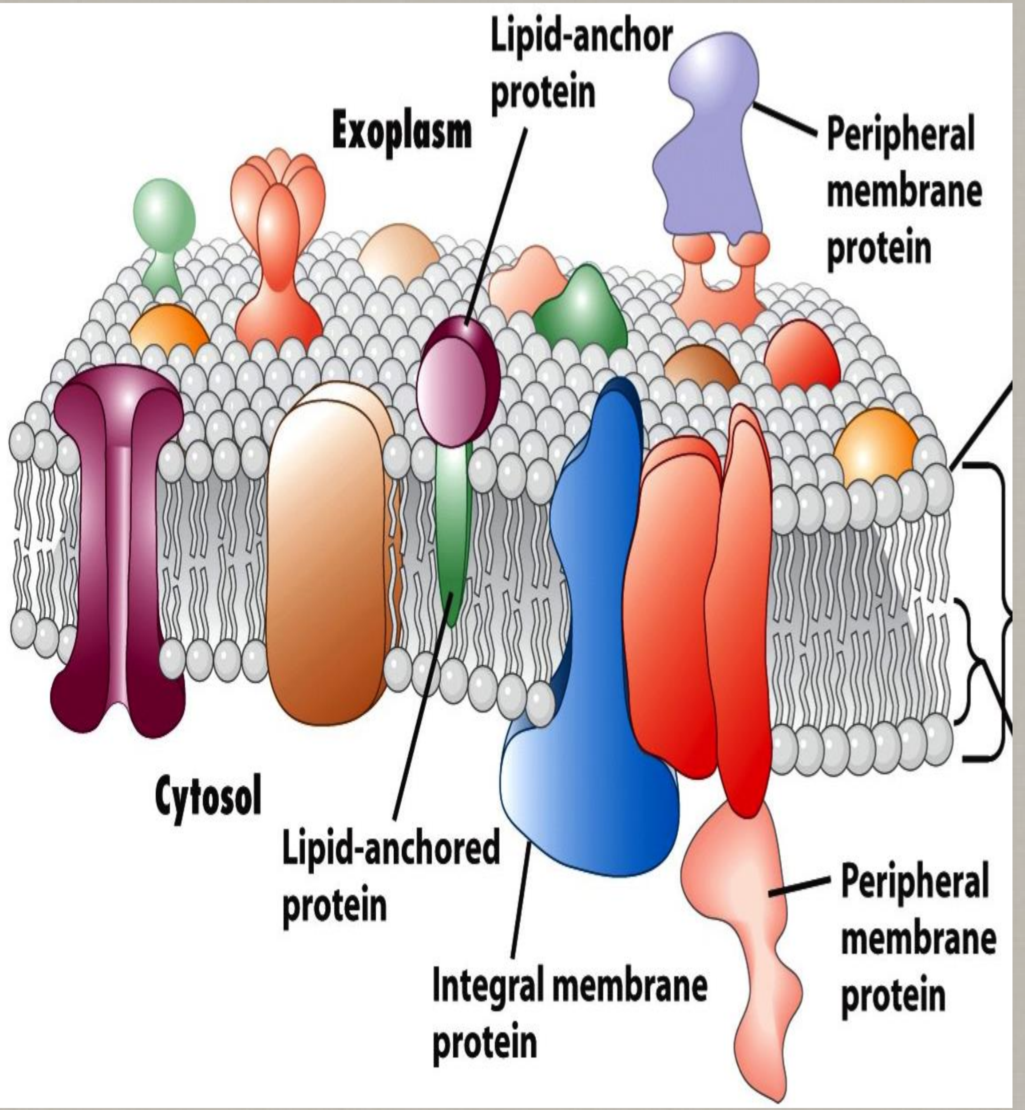
Restraints on membrane protein mobility

- Lipids
- Association with extra-membranous proteins like the **cytoskeleton**, **extracellular matrix proteins**, and others.
- Other membrane protein associations.
- Extracellular carbohydrate groups (on glycoproteins) limit proteins movement.



Membrane proteins are asymmetric

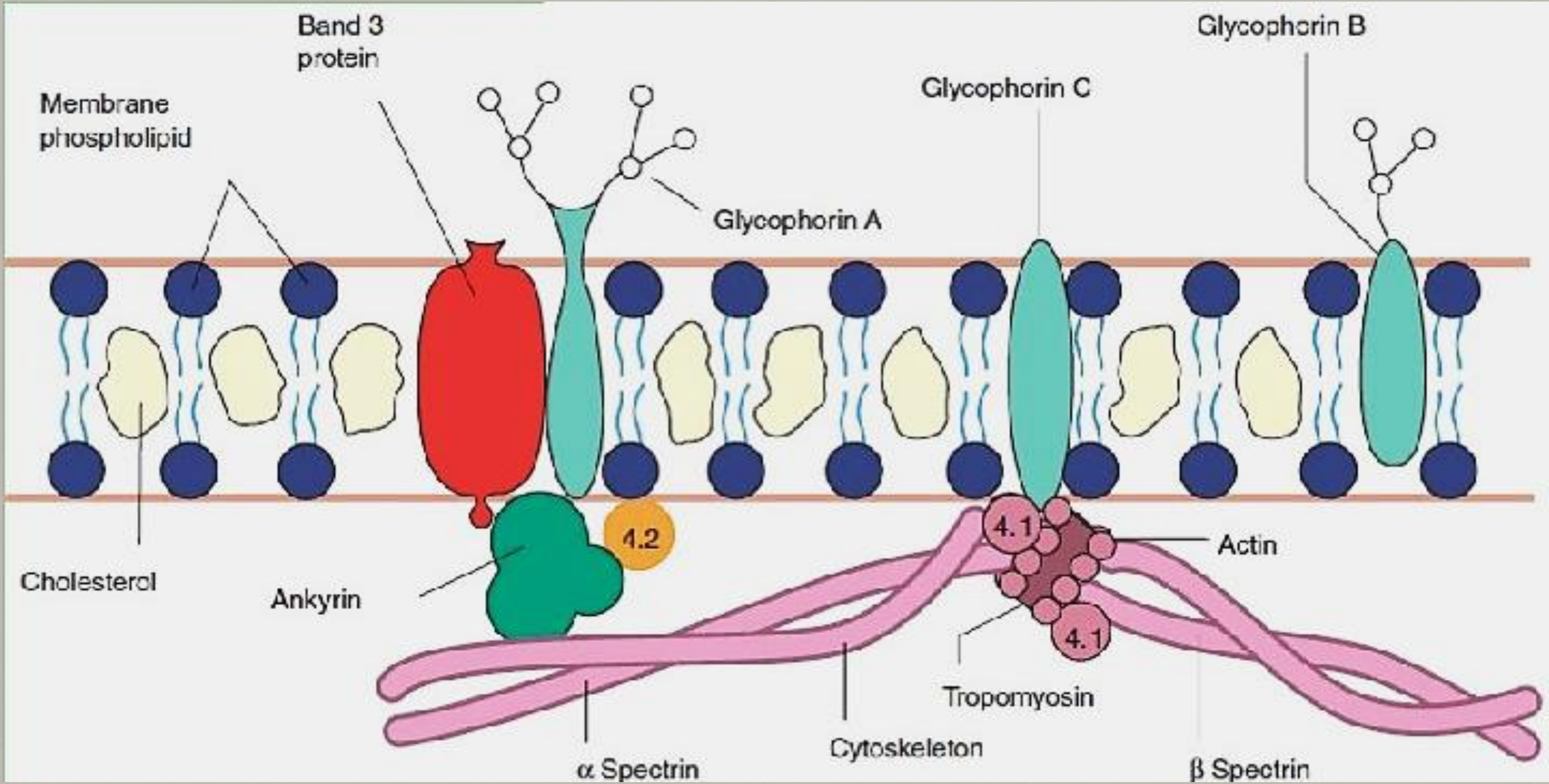
- **Receptors** have the signal receiving part on the external side of the membrane, to receive signals sent from other cells.
- **Glycoproteins** have their carbohydrate parts sticking in the extracellular side.
- **Adhesion proteins** have large external parts extending to bind external proteins.
- **Membrane enzymes** mostly have their catalytic part at the cytoplasmic side.



Erythrocyte Membrane

Erythrocytes membrane is taken as an example

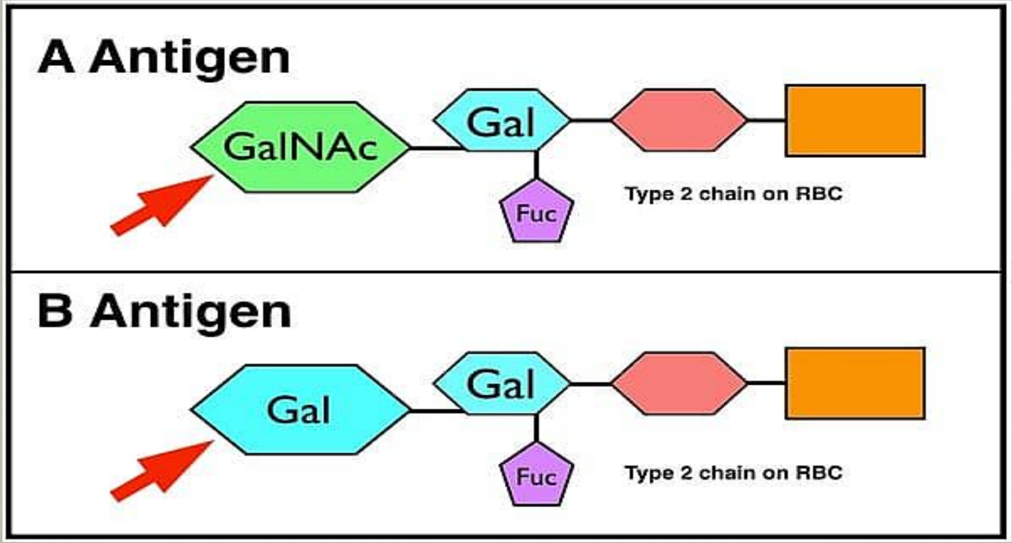
Erythrocyte membrane proteins

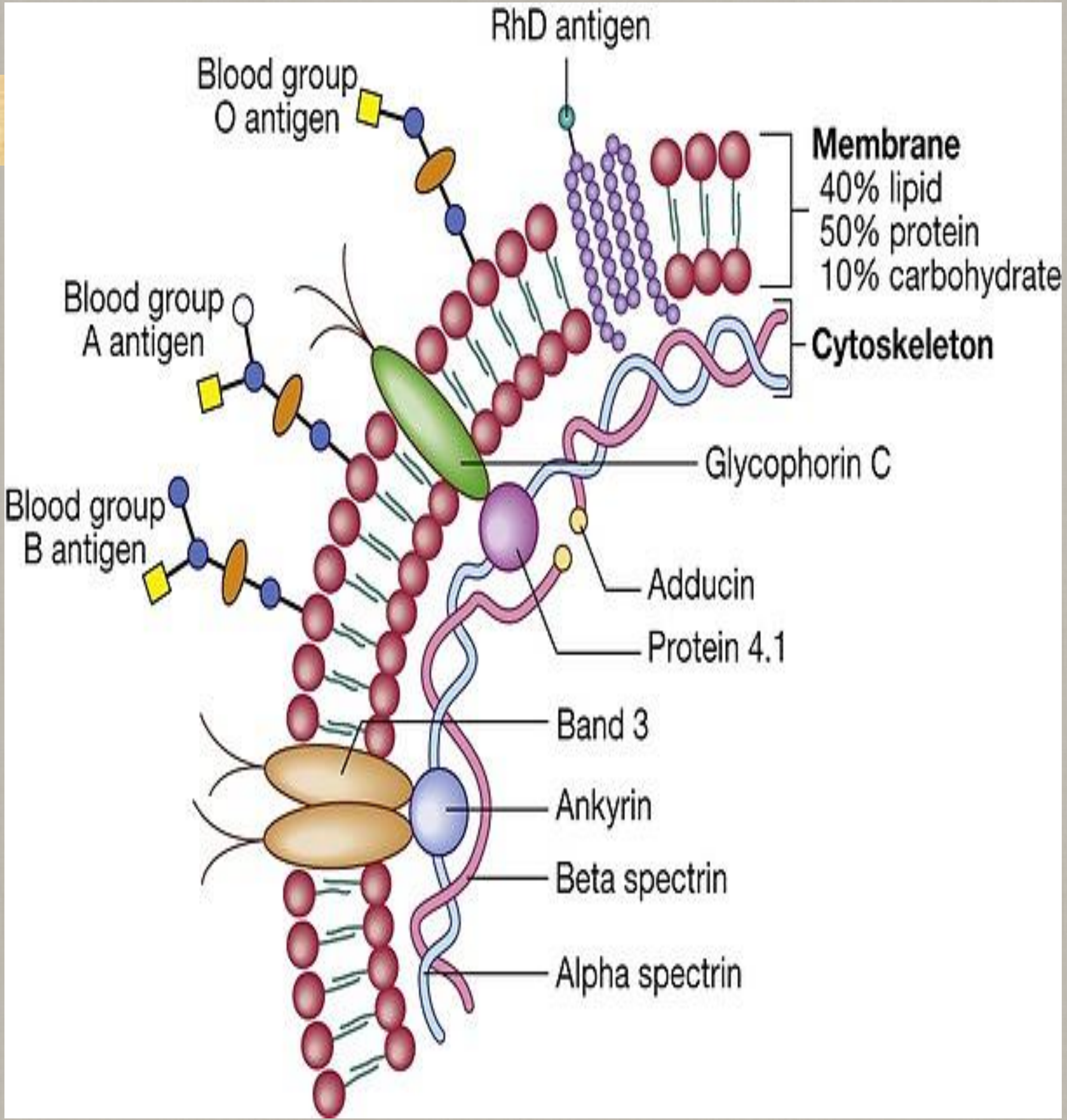


Protein	Function
Integral:	
Glycophorins	Give a negative charge to cell to reduce interaction with other cells and endothelium.
Band 3	Exchange bicarbonate ion for chloride ion.
Peripheral:	
Spectrin	Cytoskeleton protein responsible for the biconcave shape of RBCs.
Actin	Participate in protein-protein interaction.
Ankyrin	Interacts with spectrin and Bnad 3, links membrane to cytoskeleton.
Protein 4.1	Stabilizes actin-spectrin interaction
Protein 4.2	Help interaction of Band 3 protein with ankyrin

ABO Blood Group System

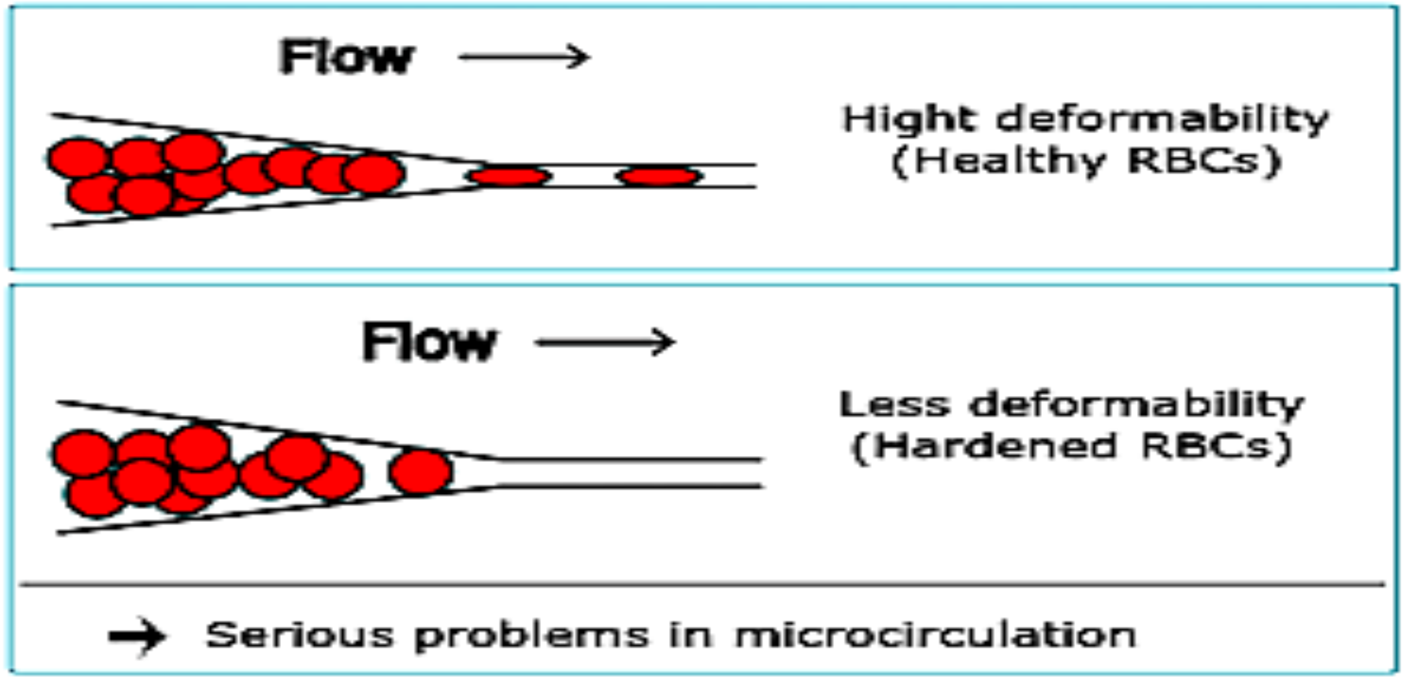
The ABO blood group system is based on antigens of RBCs. These antigens are carbohydrate units presented at the end of membrane **glycolipids**.



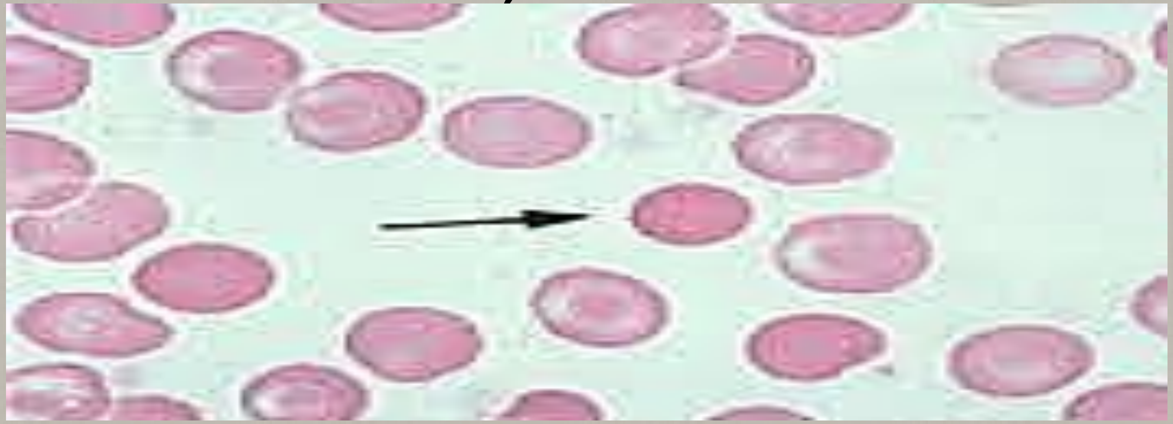


Erythrocyte deformability

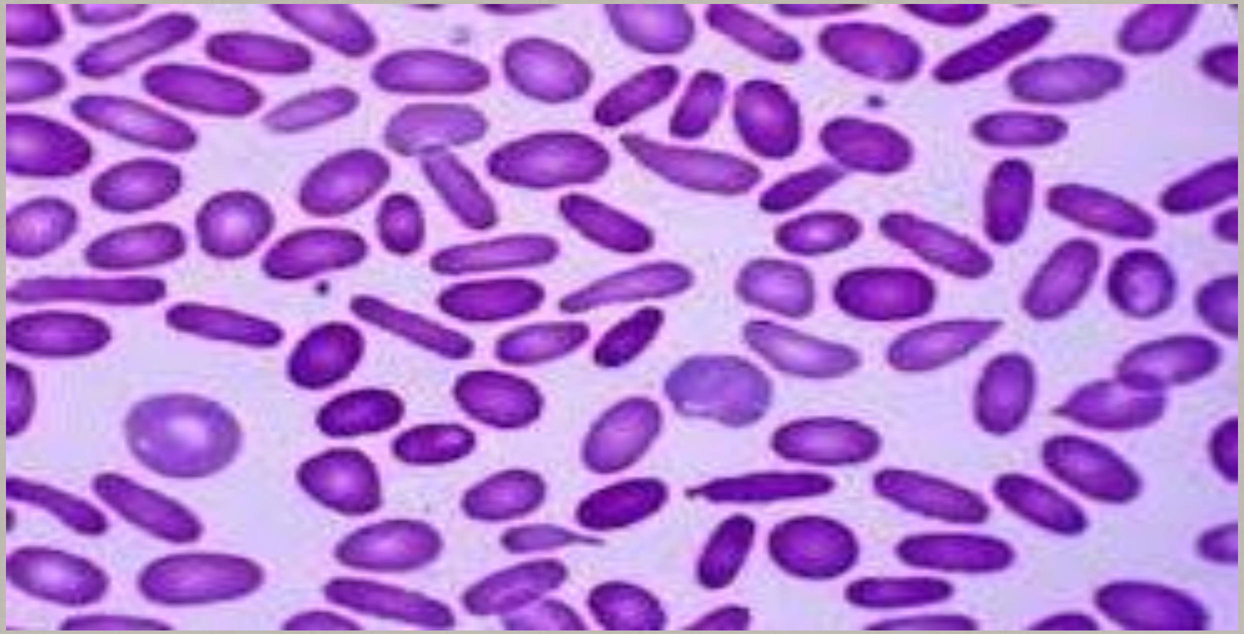
- **Erythrocyte deformability** is the ability of erythrocytes to change their shape extensively under the influence of mechanical forces in fluid flow or while passing through microcirculation, so as to avoid hemolysing (rupturing).
- This phenomenon is possible because of the membrane flexibility, and the **cytoskeleton**.



- In **Hereditary Spherocytosis**, **spectrin** levels is 40-50% depleted.
- The cells round up and become much less resistant to lysis when passing through capillaries, and when lysed they are cleared by the spleen.
- The shortened survival of RBCs and the inability of the bone marrow to compensate for them, lead to haemolytic anaemia.



- **Hereditary Elliptocytosis** is a common defect where **spectrin** is unable to form a network with actin, resulting in fragile elliptoid cells.



- Treatment with **cytochalasin** drugs, which affects actin filament, can alter the deformability of the erythrocyte.

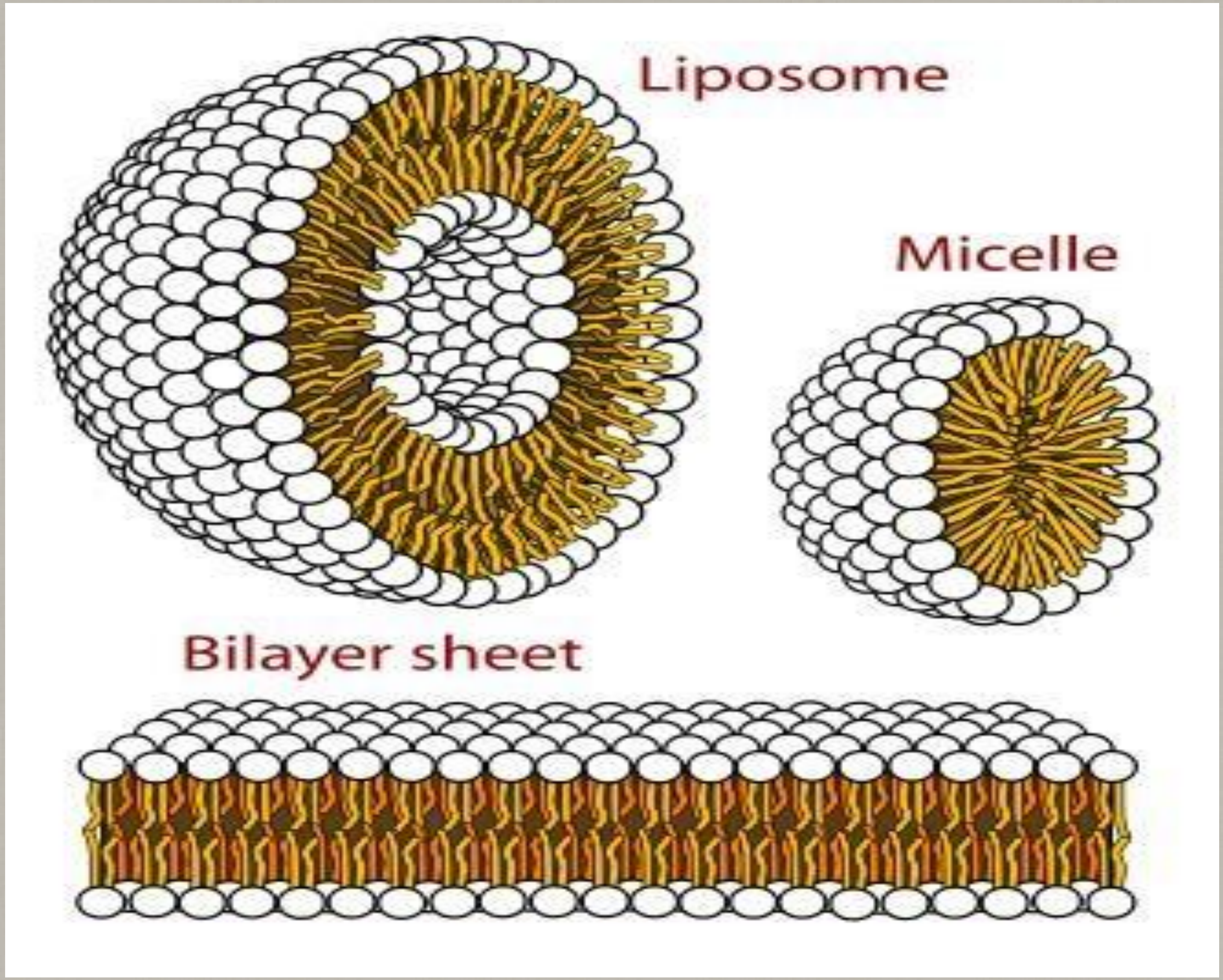
Membrane formation

Membrane Lipid synthesis

- **Phospholipid synthesis** takes place on the smooth ER.
- Fatty acids of the bilayer form van der Walls bonds.
- Electrostatic and hydrogen bonding between phosphate head groups, and between them and water, is formed.

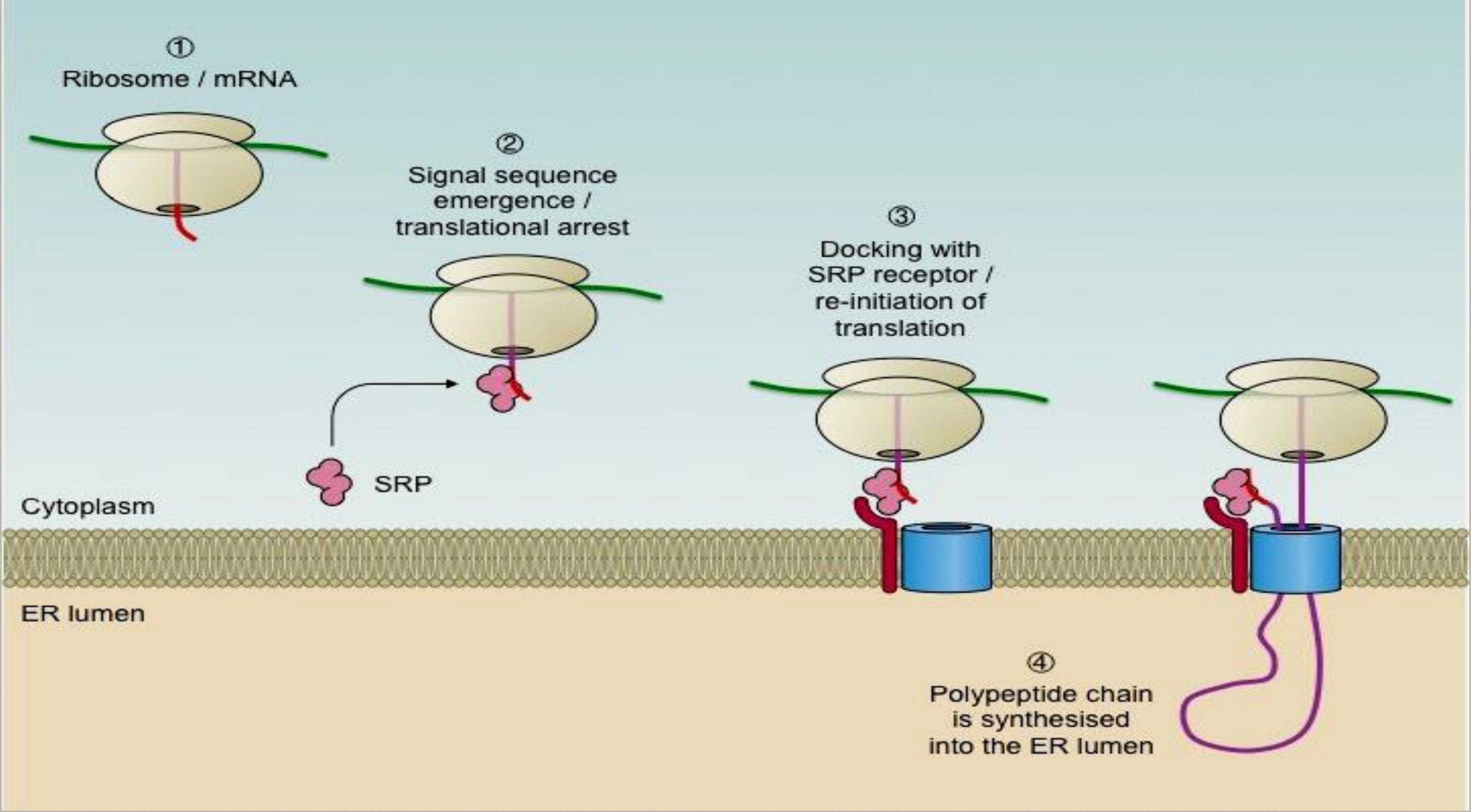
Amphipathic molecules form one of these structures (shown in the figure) in water.

In the case of the cell the bilayer is the only possibility.



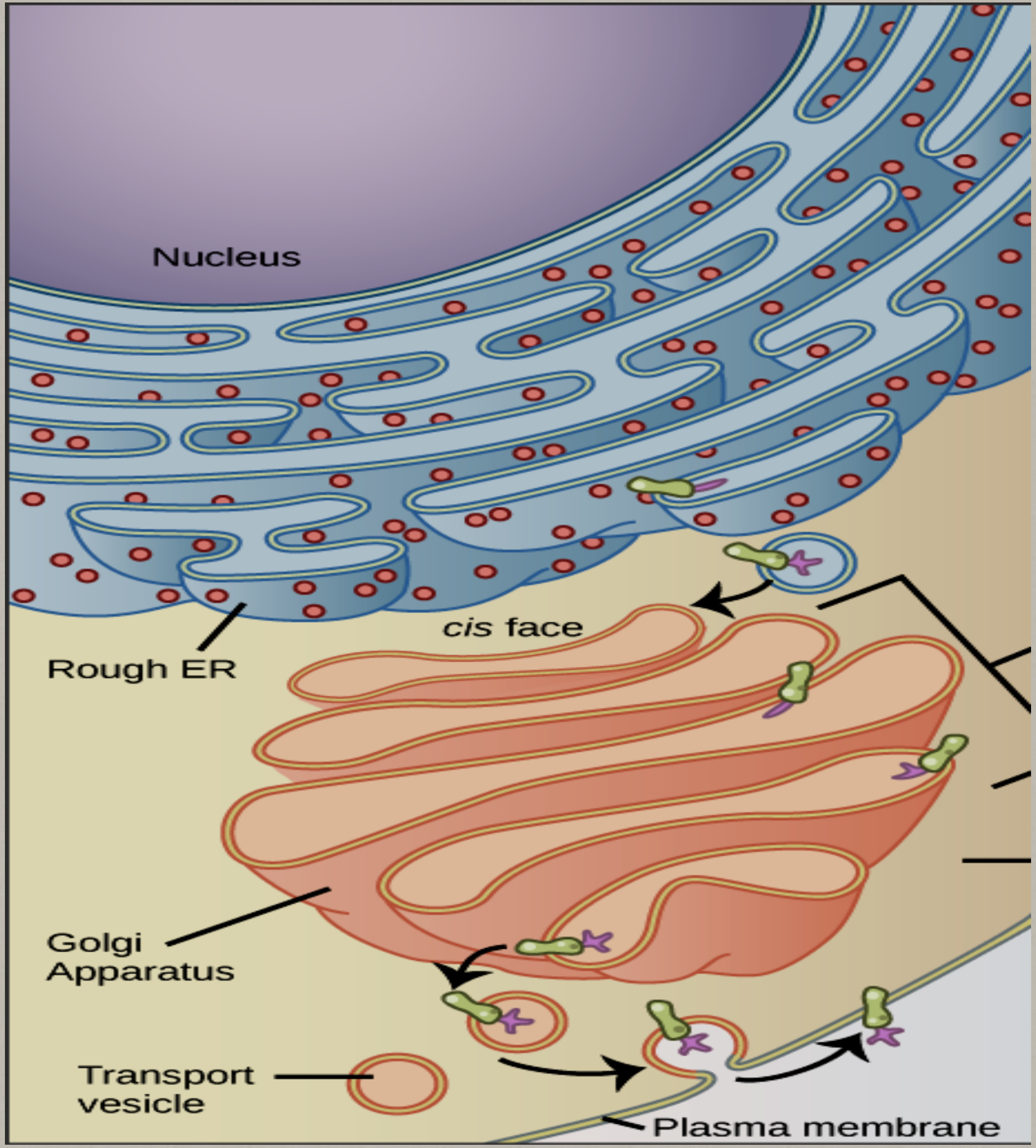
Membrane proteins synthesis:

1. At the rough ER, protein synthesis initiates on free ribosomes.
2. **Signal sequence** is produced (as part of the protein).
3. **Signal sequence** is recognized by **signal recognition particle** (**SRP**). Protein synthesis is paused.
4. **SRP** directs the ribosome to **SRP receptor** on the ER membrane, and the **SRP** dissociates.
5. Protein synthesis ***continues*** and the newly formed polypeptide is passed into the ER lumen via a **pore** in the ER membrane.


①
Ribosome / mRNA
②
Signal sequence emergence / translational arrest
SRP
③
Docking with SRP receptor / re-initiation of translation
Cytoplasm
ER lumen
④
Polypeptide chain is synthesised into the ER lumen

6. The protein is then transported via a vesicle to the Golgi complex.

7. At Golgi the protein is subjected to post-translation modifications, then delivered via vesicles to the cell membrane.



References

1. **Molecular Cell Biology**, by Harvey Lodish *et al*.
2. **Molecular Biology of the Cell**, by Bruce Alberts *et al*.
3. Wikipedia.

Some important Youtube videos

https://www.youtube.com/watch?v=zT2CCLzYZ8M

https://www.youtube.com/watch?v=WWSCd7Bouic

https://www.jove.com/science-education/10972/membrane-fluidity

https://www.youtube.com/watch?v=UxvFdW9aO0s